Spring 5-1-2015

Exercise-Induced Adult Neruogenesis and the Seizure Threshold: the Role of COX-2

Gina Kim
Syracuse University

Exercise-Induced Adult Neurogenesis and the Seizure Threshold: the Role of COX-2

A Capstone Project Submitted in Partial Fulfillment of the
Requirements of the Renée Crown University Honors Program at
Syracuse University

Gina Kim
Candidate for B.S. Degree
and Renée Crown University Honors
May 2015

Honors Capstone Project in Biology and Neuroscience

Capstone Project Advisor: _____
James Hewett, Associate Professor

Capstone Project Reader: _____
Kari Segraves, Associate Professor

Honors Director: _____
Stephen Kuusisto, Director

Date: May 5th, 2015

Abstract

Neurogenesis, the generation of new neurons, is most prevalent when the brain is being formed during pre-natal development. However, this process continues in select areas in the brain during adult life as well. One such area in the brain is the dentate gyrus (DG) of the hippocampus, an area known to be associated with learning and memory. In this region, neurogenesis is believed to contribute to neuroplasticity as well as improving its functions in learning and memory. Interestingly, this synthesis of neurons is increased by physical activity—predominantly running—and by seizures originating in the limbic system. The increased excitatory neuronal activity that occurs during a seizure leads to an increase in an enzyme called cyclooxygenase-2 (COX-2), the initial enzyme in the production of prostaglandins from arachidonic acid. COX-2 is usually expressed in certain neurons of the brain including the CA3 subregion of the hippocampus, and its level of expression is linked to increased excitatory neuronal activity. In other words, COX-2 is up-regulated by seizure activity; therefore, during seizures there is an increase in COX-2 activity and expression. Because new neurons exhibit increased excitatory responsiveness, the goal of this research is to test the possibility that exercise-induced new neurons of the DG will exhibit increased COX-2 levels following acute seizure activity. It is predicted that it will, and amplified COX-2 levels will be found in subjects that have access to physical exercise, particularly in the new neurons generated by running. An experimental group of mice were supplied with running wheels and allowed to run for roughly one month. Bromodeoxyuridine (BrdU) was injected one week after the start of running in order to label the newly generated neurons in the hippocampus. After running, animals were subjected to acute seizures through pentylenetetrazol (PTZ) injections, and the subsequent seizures were scored based on severity. Cardiac perfusions were then performed to collect brain tissue that was then coronally sectioned using the cryostat and mounted on microscope slides, in order to stain for COX-2 and BrdU. Running mice exhibited lower seizure scores than control mice implying that the neurogenesis induced by physical activity increased the COX-2 levels, thus raising the seizure threshold in exercised mice.

Executive Summary

Seizures are caused by abnormal and excessive electrical activity in the brain. People are said to have epilepsy when they have had two or more seizures in their lifetimes. Thus, epilepsy is characterized by reoccurring seizures that happen without provocation. This disease has been diagnosed in roughly 1% of the population worldwide. There are a myriad of factors that can cause seizures with the most common including infection, trauma, high fevers in children, and genetic factors. In addition, brain damage from other disorders like stroke—which deprive the brain of oxygen—may also cause seizures. However, in about half of all cases in epilepsy, the cause is unknown demonstrating much research is necessary to better understand this elusive disease. Antiepileptic drugs are by far the most common approach to treating those with epilepsy. Yet there is still no drug that cures it. There are dozens of antiepileptic drugs that for the most part, help epileptic patients maintain and manage their seizures by suppressing them. But there are full of side effects including sedation and alternatives in cognition. It is up to health care providers to tailor the right combination of drugs to each patient because everybody reacts differently to them; epileptic episodes have different triggers in different people. Consequently, any kind of drug treatment is really just trial and error, which is inefficient and taxing on the patient. In addition, epilepsy about 30% of the time, does not respond to any antiepileptic drugs at all. In more extreme cases, surgery is recommended in which surgeons remove or sever connections to the brain area where the seizures are originating. This is limited by important that brain region is for everyday behavior. None of these treatments are ideal, which is another reason why epileptic research is so important today.

Epilepsy is centered on the idea that there is a balance between excitation and inhibition in the brain, and if excitation is increased, a seizure may occur. There are two ways to increase

excitation: by increasing excitatory neurotransmitters like glutamate, and by decreasing inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA). In both situations, excitation is in excess increases the probability of a seizure. After the increased neuronal activity that occurs during a seizure, levels of an enzyme called cyclooxygenase-2 (COX-2) immediately rise in neurons of the hippocampus and cortex. COX-2 plays a key role in the metabolism of arachidonic acid, which is a polyunsaturated fatty acid that is stored in the membrane phospholipids. Arachidonic acid is freed from a phospholipid molecule by the enzyme phospholipase A2. When it is released, COX-2 can metabolize it—transforming arachidonic acid into a variety of different prostaglandins. These prostaglandins are compounds that have varying hormone-like effects that help the body with many different functions including constriction and dilation of vascular smooth muscle cells, inducing labor, and regulating inflammation, calcium movement, and hormones. COX-2 expression may be an important mechanism to suppress seizure activity and bring excitatory levels back down. Hence, this increase in COX-2 after subsequent excessive excitatory activity in the brain may be an endogenous, antiepileptic mechanism. For the purposes of this project, high levels of COX-2 are viewed as good because they are neuroprotective and keep seizure activity in check. This project focuses on neurogenesis as something that can further increase COX-2 levels, helping to suppress seizure activity. Neurogenesis, the generation of new neurons, is most active during pre-natal development. However, new neurons are also formed throughout adult life, specifically in the dentate gyrus of the hippocampus, and this is increased by exercise. These newly generated neurons exhibit increased responsiveness; they are malleable and easily influenced by changes produced by drugs. Because new neurons exhibit increased excitatory responsiveness, I predict that these neurons in particular will overreact to a seizure, and produce higher levels of COX-2 than the

regular, more mature neurons around them. This will lead to overall amplified COX-2 levels in exercised mice as opposed to non-exercised mice. In addition because I conjecture that exercised mice will have higher COX-2 levels, I also hypothesize that these animals will have a higher seizure threshold than the non-exercised animals—meaning a stronger stimulus will be needed in order to induce a seizure in these mice.

These studies could add to the accumulating evidence suggesting that voluntary exercise is neuroprotective. In addition to increasing performance in hippocampal functions, such as synaptic plasticity, learning, and memory, exercise may also increase the seizure threshold—information that could have implications for future development of treatments for epileptic patients.

Table of Contents

Acknowledgements

I would first and foremost like to thank my Principle Investigator, Dr. James Hewett. Without his endless guidance, support, and motivation, I would not be where I am today. He is truly one of the best mentors I have come to work under in my four years at Syracuse, and I am thankful that I have had to opportunity to probe his busy, intelligent, and humble mind. He has taught me to think independently as well as work as a team player and has also taught me a lot about dry humor; I still never know when he is joking. I could not have asked for a more understanding and encouraging advisor who has really opened up my eyes to scientific research. All of the successes I have had regarding my research are thanks to him.

I would also like to thank our lab manager, Miriam Gladstone. Though she has only been with us for about a year, she has transformed the productivity and organization of the lab tremendously. She has always offered me a helping hand and aided me immensely in the making of my graphs, figures, and posters. In addition, Yifan Gong, a graduate student in the lab who is probably the smartest person I know, taught and helped me greatly when I first joined the lab in my sophomore year. I have never met such a hardworking, meticulous, and inherently kind person. Finally, I want to thank all of the undergraduates who I have come to know while working in the lab: Phill Groden—who welcomed me with open arms and taught me many procedures, Ayanna Pringle—who joined the lab the same time I did and was a great friend to me, Kate Bunch—who has always been by my side throughout late nights as we tried to finish our experiments and capstones together, and all of the other students who have really made this lab like a second home to me.

Lastly, I want to the Syracuse University Biology Department as well as the Renée Crown Honors Program for funding my research and my parents who motivate me and support me in every way possible, in every sense of the word.

Chapter 1

Introduction

Epilepsy

Epilepsy is a common brain disease that is characterized by recurrent spontaneous seizures[1]. Affecting approximately 1% of the worldwide population[2], epilepsy is a disease that has no racial, geographical, or social class boundaries and is remarkably uniformly distributed around the world[1]. Derived from Latin and Greek words for "seizure" or "to seize upon", epilepsy is truly an ancient disease that has been recorded in documents dating over 3000 years[1]. It was largely thought to be a supernatural disease where those affected suffered from an invasion of an evil spirit until Hippocrates challenged this idea in the 5th century. His concepts of epilepsy being a treatable brain disorder began to take root in the 17th century[1]. Today there are still cultures and civilizations that believe in the spiritual side of epilepsy including the Hmong and practitioners of voodoo. Though modern science has made leaps and bounds in the expansion of knowledge of epilepsy, there remains more to learn.

The two main seizure types include partial or focal seizures and primary generalized seizures. Where the former show seizures from a localized area within the cerebral hemisphere, and the latter show abnormal activity in both sides of the brain[2] (**Tab. 1**).

Partial Seizures	Simple • with motor signs • with somatosensory or special-sensory symptoms • with autonomic symptoms or signs • with psychic symptoms (disturbance of higher cerebral functions)
	Complex • without automatisms • with automatisms • with impairment of consciousness at onset without automatisms
Generalized Seizures	Absence • mild clonic, atonic, tonic, or autonomic activities, or automatic behavior
	Myoclonic
	Clonic
	Tonic
	Tonic-clonic
	Atonic

Table 1. Outline of the International Classification of Epileptic Seizures[1].

Focal seizures can be further divided up into simple seizures and complex seizures. Focal seizures are largely distinguished by the area of the brain in which they originate. A person experiencing simple focal seizures will stay conscious and may experience unexplainable changes in mood. They are also said to hallucinate motor and sensory stimuli, such as seeing or smelling things that are not real[3]. Though people experiencing complex seizure are conscious, they are in an altered form of consciousness described as a dreamlike state. During this state people will often display strange repetitive behaviors called automatisms—like walking around in circles or rapid blinking— and will usually have no recollection of doing so doing throughout

the episode[1]. Seizures such as these only last a couple of minutes and people who are affected by this can usually sense the onset of a seizure[3]. Focal seizures are often confused with other disorders such as narcolepsy, fainting, or even mental illness[3]. Generalized seizures are a result of a seizure that has affected both of the hemispheres of the brain and is characterized by a loss of consciousness with or without massive muscle contractions. These seizures can be further broken up into absence seizures, tonic seizures, clonic seizures, myoclonic seizures, tonic-clonic seizures, and atonic seizures[2]. An absence seizure is characterized by a person remaining still and staring off into space. A tonic seizure is when a person will stiffen up the main muscles of the body and a clonic seizure occurs when that person experiences repeated jerking movements of their muscles. During a myoclonic seizure, a person will jerk or twitch. A tonic-clonic seizure is the widely popularized form of seizures in the media and demonstrate a person stiffen up their body as well as having repeated full body jerks while losing consciousness. Finally, an atonic seizure is characterized by a loss of muscle tone making a person fall down or droop their head[3]. After experiencing a seizure, people fall into a post-ictal state during which they may feel tired, sleepy, weak, or confused[3].

The root of this wide variety of symptoms lies in the functioning of the brain. The foundation of epilepsy is grounded in the idea that there is a balance between excitation and inhibition in the brain, and if the excitation is increased, a seizure occurs. At the start of a seizure if neurons start to signal abnormally at the same time, a great deal faster than normal and as many as 500 times a second[3], this surge of excitation may cause seizures. There are two seizure models based on ways to increase excitation in the brain: by enhancing excitation or by blocking inhibition. Chemically, if excitatory neurotransmitters such as glutamate are increased in the brain, a seizure will occur. Glutamate fires excitatory post synaptic potentials that depolarize

neurons and make it more likely for a neuron to fire an action potential. Alternatively, if inhibitory neurotransmitters such as GABA are decreased in the brain, a seizure may occur. GABA causes inhibitory post synaptic potentials and decrease excitability making it more difficult for a neuron to initiate an action potential. When this is blocked, as it is in some epileptic patients, the balance is shifted and there is too much excitation.

The broad spectrum of symptoms that Epilepsy elicits hints at its many different causes and treatments. 50% of the people who are diagnosed with epilepsy never discover the cause of the disease[3]. The main causes include infection—such as meningitis and HIV, traumatic brain injury, brain tumors, genetic factors, other diseases such as stroke—that deprive the brain of oxygen, and high fevers that can elicit febrile seizures in children[3].Although this disease has no cure, there are many ways to control seizures—the most common approach is using antiepileptic drugs (AEDs) that suppress the seizures with a sedative like effect. There are more than 20 different AEDs available today all with different advantages and a great deal of side effects[3]. In a trial and error kind of treatment, a doctor must tailor the right combination of AEDs to patients depending on a number of factors because everybody reacts differently to them; epileptic episodes have different triggers depending on the person. This may take several months to determine the best drug and dosage, and 30% of the time, AEDs do not work at all. Medication as a method of treatment is frequently inefficient and taxing on the patient[3]. Other techniques, though not as common, also exist in the management of epilepsy such as dietary approaches. The ketogenic diet, consisting of a high-fat and low carbohydrates, forces the body to break down fats instead of carbohydrates to survive and has been used mostly in children[3]. This diet, however, has only cured the disease 10% of the time and is extremely hard to maintain, with side effects including nutritional deficiency and impaired growth[3]. If noninvasive treatments fail,

surgery can be performed as a last effort to treat severe epilepsy. In this procedure, surgeons sever connections in the brain in order to stop the spread of seizure activity or remove the area of the brain where the seizures are originating—depending how important this area of the brain is for daily behavior. They usually try to avoid areas that are necessary for speech, movement, sensation, memory and thinking[3]. In the famous case of Henry Molaison, he had his hippocampus surgically removed in order to combat his epilepsy. Following the procedure, he suffered from severe anterograde amnesia. This case study showed just how important the hippocampus was for learning and memory and how large the risk is for procedures such as these. None of these treatments are ideal which is why epileptic research is important today.

Cyclooxygenase-2

Shortly following the increased neuronal activity after a seizure, an enzyme called cyclooxygenase-2 (COX-2) has been found to be induced rapidly in specific areas of the epileptic brain¯primarily in the hippocampal formation, which is made up of the dentate gyrus (DG), the carbonic anhydrase III (CA3) and the carbonic anhydrase I (CA1), with mossy fibers connecting the DG to the CA3 and Schaffer collaterals connecting the CA3 to the CA1[8]. So when excitatory levels go up, increased activity and expression of COX-2 levels shortly follow. COX-2 plays a key role in the metabolism of arachidonic acid, which is a polyunsaturated fatty acid that sits in the membrane phospholipids[4]. When arachidonic acid is released, COX-2 metabolizes it, transforming it into a variety of different prostaglandins, which are compounds that have varying hormone-like effects and help the body with many different functions. COX-2 is constitutively expressed in excitatory hippocampal neurons and is regulated strongly by synaptic activity and less, if not at all, expressed in the inhibitory neurons in the hippocampus[4].

This increase in COX-2 levels subsequent to seizure activity is a protective, antiepileptic mechanism[4,5,6,7]. COX-2 in the post-ictal period serves to suppress the seizure activity and bring excitatory levels down. In other words, COX-2 is up-regulated by seizure activity (**S1**[8]). So essentially, high levels of COX-2 are good. COX-2 is neuroprotective and keeps seizure activity in check. Turrin et al. (2004) and Vezzani (2005)'s work compared constitutive COX-2 mRNA expression in a normal mouse brain and in a mouse brain that had a seizure. Their findings showed that the mouse subjected to a seizure had much more COX-2 labeled neurons in its DG, illustrating that COX-2 expression indeed enhances in the hippocampus after convulsive seizure activity[5,6].Not only does COX-2 mRNA expression rise following an acute seizure, but protein expression rises as well[7].Claycomb et al. (2011) compared three images of COX-2 stained hippocampi from different mice: one that was given Saline, one that was given a drug induced seizure three hours prior to tissue collection, and one that was given a drug induced seizure 72 hours prior. The control mouse that was not given a seizure showed fairly low COX-2 levels[7]. On the other hand, the mouse that was subjected to a seizure showed a great increase in COX-2 in the hippocampus[7]. Finally, in the third mouse, COX-2 levels decreased and returned to baseline after 72 hours past, now comparative to a mouse that had no saline[7]. This indicates that convulsions elicited a rapid and transient increase in the COX-2 protein expression in the hippocampus[7]. In addition, the expression of COX-2 was increased more in the DG of the hippocampus than the CA3 subregion[7]. All studies indicate that COX-2 expression increases substantially after a seizure.

It is interesting to note that while it is widely agreed that COX-2 increases with the event of a seizure, many are not in agreement as to the reason why. While this report asserts that COX-2 is an antiepileptic mechanism that serves to bring seizure activity down, there are others who

think oppositely in that the increased COX-2 levels are making seizures occur in the first place. Claycomb's paper goes on to reject those that think COX-2 inhibitors could treat epilepsy[7]. This article challenges claims that declare COX-2 has a worsening effect on seizures by affirming that suppression of COX-2 is actually deleterious to the epileptic brain[7]. Therefore, COX-2 inhibitors would be an ineffective treatment for epilepsy, further demonstrating its antiepileptic properties.

Neurogenesis

This paper focuses on neurogenesis as something that can further increase COX-2 levels in the brain, helping to suppress seizure activity. Neurogenesis is the generation of new neurons and is most active during pre-natal development. However, it has been recently found that this process also continues throughout adult life—specifically in the DG of the hippocampus. Adult neurogenesis originates from neural progenitor cells (NPCs) in the subventricular zone (SVZ) and the subgranular zone (SGZ), differentiate and mature into adult-born neurons, and then integrate in the neural network of the hippocampus mostly as dentate granule cells (DGCs)[8]. The adult-born DGCs continue to develop at week one when they extend their dendrites in the granule cell layer (GCL) and the molecular layer (MOL), and extend their axons toward the CA3[9]. At week three, DGCs start to receive glutamatergic input from the perforant pathway and both the efferent and afferent synapses begin to form[9]. Finally at about two months of age, the DGCs are almost indistinguishable from mature DGCs[9]. The new neurons are integrated into the hippocampus and provide the network with the plasticity necessary for learning and memory. Because all of these processes of neurogenesis are occurring in the hippocampus, it is not surprising that neurogenesis is regulated by factors associated with the hippocampus such as learning and memory and physical exercise[8].

Interestingly, exercise is able to increase the generation of new neurons in the adult hippocampus, promoting learning and memory[8,9,10]. Exposure to physical exercise is known to regulate the cellular properties of the hippocampus due to the increased neurogenesis,[9] meaning physical exercise, such as running, increases neurogenesis. Pragg et al. (1999)'s experiment depicts just how much running increases the neurogenesis in the dentate gyrus of the hippocampus as compared to other controls[10]. This infers that cell proliferation does in fact increase in mice given unrestricted access to voluntary running. Exercised-induced neurogenesis may go on to further improve learning as well[11]. So not only can exercise modulate and increase the process of neurogenesis in the hippocampus, which continues in the adult brain, exercise is able to boost that area of the brain and improve learning and memory. In Praag (1999)'s work, he tested a control group and a runner group of mice that was subjected to voluntary running. Both groups were put through the Morris water maze task in which they must swim throughout a maze and onto a platform. Animals learn to find the platform after several trials. The runners performed much better than the controls and were able to get to the platform with decreased path length and latency—so overall faster[12]. Ergo, running enhances acquisition of the water maze memory task due to a higher quantity of new neurons. In addition, factors that reduce neurogenesis—such as corticosterone treatment, stress, and aging—are associated with diminished performance on spatial learning tasks[12]. These newly generated cells may mediate increased synaptic plasticity and improve learning[12].

Hypothesis

Because exercise, such as running, induces neurogenesis in the hippocampus, and COX-2 levels are up-regulated by seizure activity to serve as an antiepileptic mechanism, and finally

because new neurons are malleable, aid in plasticity, and exhibit increased excitatory responsiveness—I predict that these new neurons in particular will overreact to an acute seizure and produce higher levels of COX-2 compared to the older neurons around them. This will lead to overall amplified COX-2 levels in exercised mice as opposed to non-exercised mice. Moreover, because exercised mice will have higher COX-2 expression in their hippocampi, I also hypothesize that runners will have a higher seizure threshold than the non-exercised animals—meaning that a stronger stimulus will be needed in order to induce a seizure in the running mice. This would imply that voluntary exercise could increase the seizure threshold via increasing COX-2. If this hypothesis is supported in my results, this information could be used to develop new therapies for epileptic patients in the future.

Chapter 2

Methods

Model Organism

The model organism used in these studies was *Mus musculus,* or the mouse. Rats and mice combined constitute roughly 90% of the total animals used for all research purposes and the reasons why are evident[13]. There are many advantages for using mice for research. First, there are a myriad of different strains of mice due to mutations, knockouts, and transgenic mice. In addition, they are easy to care for and handle and relatively inexpensive to maintain in the lab. Mice have a very high reproductive performance with large litter sizes and short gestation periods. Thus, many generations can be produced in a short amount of time (about 1 million descendants after 425 days)[13]. Finally, the most important advantage in to using mice as model organism in research is that 95% of their genome is similar to humans[13]. Therefore, their anatomy, physiology, and genetics are comparable to that of humans.

Male, six week old CD-1 mice were purchased from Harlan Laboratories and housed in pairs. Upon arrival to Syracuse, they were allowed a one week adjustment period in the vivarium facility before being placed in specialized housing. Mice were allowed both food and water *ad libitum* throughout the study, meaning that they were supplied with a constant source of food and water, eating and drinking upon will. All animal protocols were approved by the Syracuse University Institutional Animal Care and Use Committee (IACUC).

Specialized Housing

Six Lafayette Instrument Mouse Activity Wheel Chambers (Model 80820F) equipped with a removable Activity Wheel Counter were used (**S2**). Every Wednesday, the cages were switched out for a set of six new cages in order to clean the dirty used ones. The number of wheel revolutions was recorded and reset daily for approximately 28 days. The average kilometers ran per day in running cages was calculated based on one revolution equating .4 meters. In each cage, the mice ran about 30,000 wheel revolutions, which is roughly 12 kilometers a day. Four cages with wheels were used as experimental running cages and two cages were used as control cages. In the first few experiments, the control cages contained a locked wheel within the cage, but for subsequent experiments I removed the wheel entirely from the control cages; this will be addressed in the Discussion.

Bromodeoxyuridine

Bromodeoxyruidine (BrdU) is a reagent that labels neurons in the S phase of cell division. It was used to permanently label all of the newly generated neurons due to the neurogenesis caused by running. An amount of 0.4 milliliters (mL) of BrdU was administered through an intraperitoneal (IP) injection to each mouse, one week after the mice started running.

Pentylenetetrazol

Pentylenetetrazol (PTZ) was the drug used to induce acute seizures. It is a $GABA_A$ inhibitor and works by blocking inhibitory pathways, thus causing excessive excitation and a subsequent seizure to occur. After completion of the exercise period for 28 days, IP injections of

PTZ were administered to each of the animals. PTZ was fresh before injections. It was kept in the -20˚C freezer, thawed, and then diluted with saline. The concentration of PTZ was altered throughout the course of the study. Initially 50 mg/kg of PTZ was administered, but then later on this dose was lowered to 42 mg/kg—as will be addressed in the Discussion. After induction, seizure activity was scored using a number system as follows.. A score of 0 is normal behavior. The mice can been seen scurrying around their cage and often grooming themselves. They are seldom ever still for a very long. A score of 1 is hypomobility in that the mice will be seen to sit in a corner of the cage and not move. They will seemingly stare off into space for long periods at a time in a zombie-like state and stop their normal behaviors of exploring the cage and grooming themselves. A score of 2 is characterized by myoclonic jerks. These mice will have 2 or more jerks that simply look like large twitches. These twitches usually occur periodically with no seizure behavior in between. Scores of 3s and 4s are classified as convulsive seizures, in which the animal will have a generalized convulsion of their whole body. A 3 is a convulsion in which the animal still has its righting reflex and remains on all 4s throughout the convulsion, whereas a 4 is the most severe seizure on this scale in which the animal will exhibit a convulsion that has made it fall over (**Tab 2**).

Stage 4	Generalized convulsion with loss of righting	Convulsive seizures
Stage 3	Generalized convulsion with righting reflex	
Stage 2	Myoclonus	Non-convulsive seizures
Stage 1	Hypomobility	
Stage 0	Normal Behavior	

Table 2. Seizure Scoring System.

Cardiac Perfusion

Three hours after the PTZ injections, tissue was harvested using a cardiac perfusion surgery. Animals were first anesthetized with a ketamine and xylazine IP injection. After the heart was exposed, a small cut in made in the right atrium. Then 20 mL of phosphate buffered saline (PBS) is injected into the left ventricle of the heart so that all of the blood naturally flows throughout the body of the mouse and out of the cut made in the atrium, leaving the brain free of blood (**S3**). A one liter stock of PBS is made using 750 mL of dionized water, 2 g of KCl, 80 g of NaCl, and 2.4 g of KH2PO4. After the PBS, another 15 mL of 4% Paraformaldehyde (PFA) is injected in order to fix the brain so that it remains true to shape throughout the subsequent processes. PFA is also made in the lab and can be used up to one week if stored in the 4˚C freezer. The PFA is dissolved in 100 mL of dionized water. 8 g are added to the heated water and a stirring rod is used to mix the suspension. This mixture is then heated until it is hot to the touch, but not boiling (60˚C). NaOH is then added drop by drop until the PFA is in solution and then the solution is then cooled and filtered. Next, 20 mL of 10X PBS is added. The pH probe is then

used to adjust the PFA solution to a 7.3 pH level. HCl and NaOH are added in minute increments to make this possible. Finally more dionized water is added in order to get the total amount of 4% PFA solution to be 200 mL. After the brain is perfused, the entire brain is carefully removed and submerged in PFA overnight. The next day, the brains are rinsed with PBS and then submerged again in 15% sucrose overnight. The following day, the brains are rinsed with PBS again and very lightly blotted using filter paper. Brains are placed in molds that are then filled with optimal cutting temperature compound (OCT). This compound surrounds the brain, suspending it and freezing it into a white block that will be used to slice into sections. The block is snap frozen using dry ice and 100% ethanol and stored in the -80˚C freezer.

Cryosectioning & Immunohistochemistry Staining

The tissue frozen in OCT can now be sectioned and plated on slides using a machine called the Cryostat. The cryostat is temperature controlled so that the brain remains frozen in the OCT and can be cut into very thin coronal sections so that it can be mounted on a microscope slide. Every third section of the entire hippocampus is mounted in 14 micrometer thick sections. The slides are then stored in the -20˚C freezer. Next, immunohistochemistry was used to stain the slides for COX-2 and BrdU and the slides were examined using a high powered microscope.

Chapter 3

Results

Study 1

Experimental running mice showed less severe seizures (i.e., lower seizure score) than the control mice in locked wheels. The seizure scores of the first couple trials of running and control mice after given 50 mg/kg PTZ injections **(Figure 1).** Each black dot and square represents one mouse. The experimental running mice showed less severe seizures than the control mice in locked wheels due to the proliferation of neurons in the DG. Running mice had a higher seizure threshold than control mice (**Figure 2**). Only 50% of the running mice exhibited severe convulsive seizures, whereas 100% of the non-running mice had convulsive seizures. However, the control was changed to no wheel in the cage due to the fact that the wheel itself causes enrichment in the control mice.

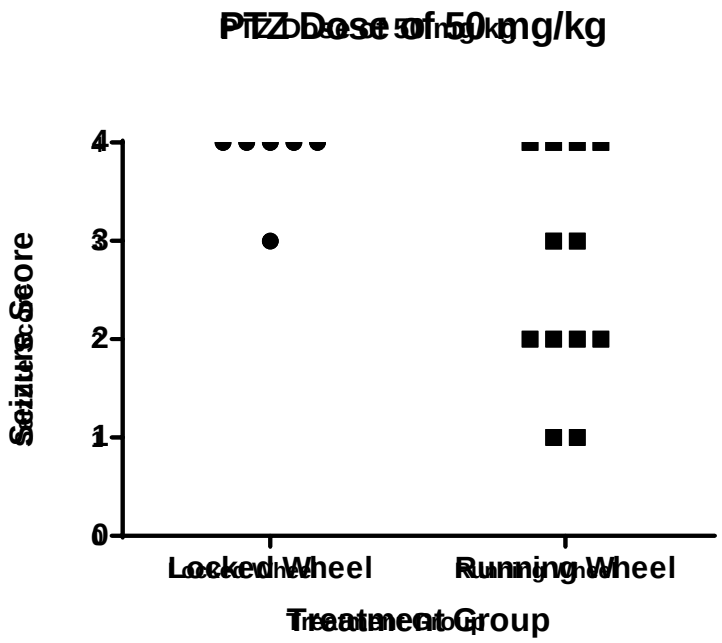


Figure 1. Effect of Running on Acute Seizures with 50 mg/kg PTZ Dose and Locked Wheel as Control.

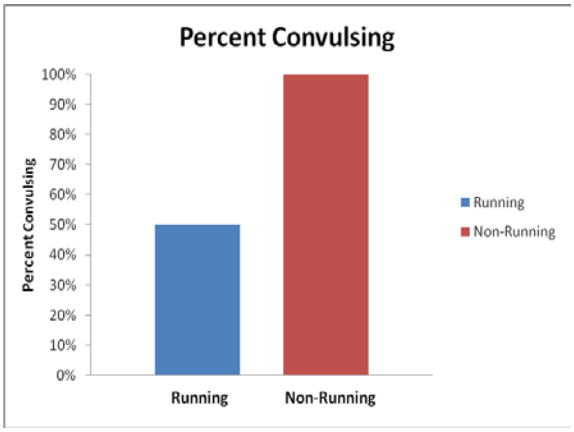


Figure 2. Effect of Running on Acute Seizures with 50 mg/kg PTZ Dose and Locked Wheel (Percent Convulsing).

Study 2

Nearly all the mice in both the control and experimental groups had a severe convulsive seizure, with the exception of one from each group—suggesting that the PTZ dose may be too high (**Fig 3**). Both groups displayed abnormally high incidence of convulsive seizures. 90.9% of running mice and 87.5% of non-running mice exhibited convulsive seizures (**Fig 4**). These percentages are very high indicating that the dosage of PTZ may be too strong.

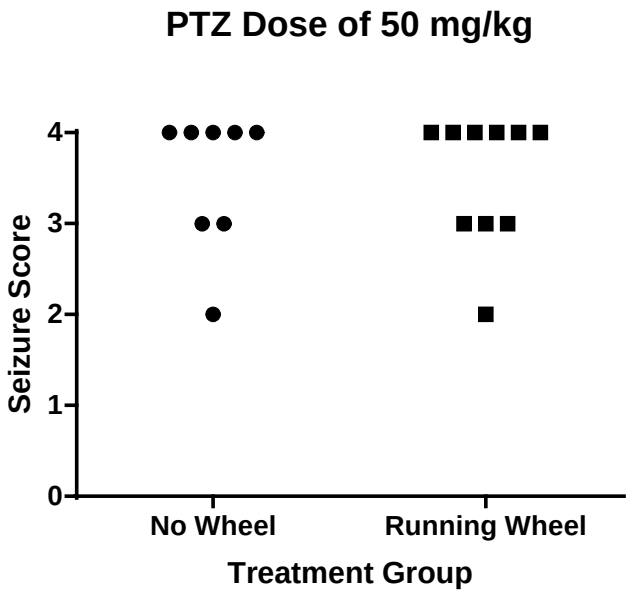


Figure 3. Effect of Running on Acute Seizures with 50 mg/kg PTZ Dose and No Wheel.

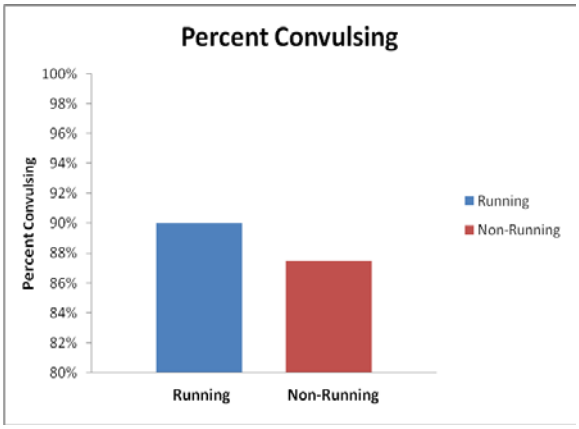


Figure 4. Effect of Running on Acute Seizures with 50 mg/kg PTZ Dose and No Wheel (Percent Convulsing).

Study 3

Running mice displayed lower seizure scores than non-running mice (**Fig 5**). The mode seizure score of control mice was 4 (horizontal bars), whereas the mode seizure score of running mice was 2. Mice that had access to running wheels exhibited more neurogenesis in the DG of their hippocampi, further implying that runners also had higher COX-2 levels and raised their seizure threshold. Only55% of the running mice exhibited a convulsing seizure whereas 82% of animals in the control group did (**Fig 6**).

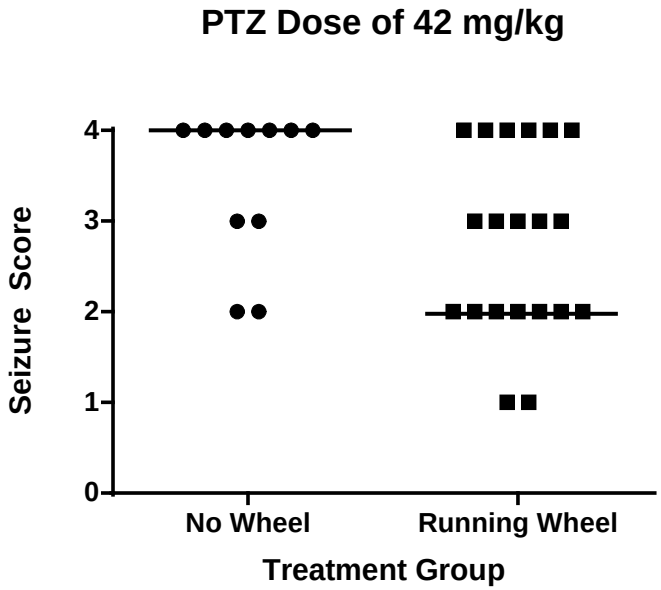


Figure 5. Effect of Running on Acute Seizures with 42 mg/kg PTZ Dose and No Wheel.

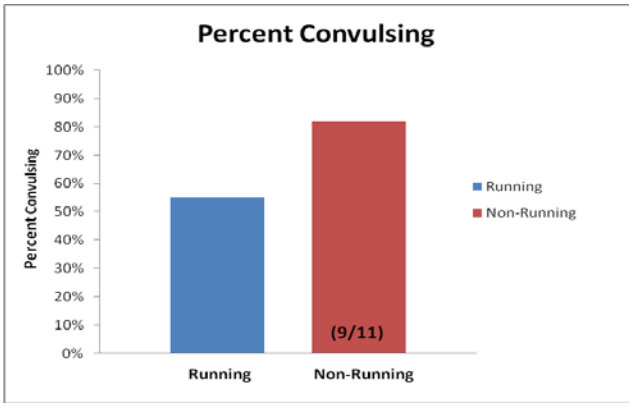


Figure 6. Effect of Running on Acute Seizures with 42 mg/kg PTZ Dose and No Wheel (Percent Convulsing).

			Cage ID					
	Date	**Day**	**A**	**B**	**C**	**D**	**E**	**F**
Week 1	**3/19**	**W**	0	0	0	0	0	0
	3/20	**R**	28649	29722	28185	30717	0	0
	3/21	**F**	30233	28226	27469	32359	0	0
	3/22	**S**	28357	31527	31858	39853	0	0
	3/23	**Su**	29963	30172	33255	44732	0	0
	3/24	**M**	31848	29640	34169	49240	0	0
	3/25	**T**	30887	32348	34544	46395	0	0
	avg		29989.5	30272.5	31580	40549.3333	0	0

Table 3. Running Wheel Data for One Week in Four Running Cages (A-D) and Two Control Cages (E-F). Mice ran roughly 12 kilometers a day.

Chapter 4

Discussion

Problem Solving

The scores look exactly how I hypothesized they would in my first few trials with 50 mg/kg of PTZ and the locked wheel as a control (**S1**)—with the running mice having lower scores of ones and twos and the control mice having more severe seizures of threes and fours. However, the control used for these trials may have not been the best one. It has been shown that even a locked wheel being in the cage could exhibit enrichment in the control mice[14,15]. When mice were provided with housing enriched with a locked wheel in Bednarczyk et al. (2011)'s paper, these mice exhibited increases in proliferation in the DG that were equivalent to those mice housed with an unlocked running wheel[15]. The locked wheel seems to represent a source of cognitive stimulation because the mice are able to climb on the wheel, even though they are unable to run on it. Stimulating these mice with an enriched environment helps reduce stress and anxiety and has been documented to increase neurogenesis[15]. Items such as these in the cage are indeed increasing the enrichment in the DG of these mice. Tashiro et al. (2007)'s paper demonstrates that the DG of mice that had nothing in its cage has lower levels of new neurons compared to the DG of a mouse that had an enriched cage[15]. It is apparent that there are much more BrdU-stained neurons in mice with enriched cages, entailing that the enhanced environment did in fact cause an increase in neurogenesis[15]. Due to these numerous studies, it

was decided that in future trials and experiments, the wheel would be taken out completely in the control, non-running cages.

In the next few trials, the wheel was removed from control cages and a PTZ dose of 50 mg/kg was used (**S3-S4**). Because a high number of mice exhibited convulsive seizures in the control group, it was decided that the concentration of the PTZ seizure convulsant used in this study was too high. In order to choose the right dose, a PTZ dose response curve was made (**S4**). Various doses were tested and the subsequent seizure score was documented. It can be seen that the 50 mg/kg concentration of PTZ that had been initially used caused all of the animals to get a score of 4, showing again that this concentration was indeed too high. Normally, it is ideal and best to see a range in scores when treating control animals with PTZ—as it is shown in the 42 mg/kg concentration of this graph—so that the results of any experimental groups can be seen to go higher or lower than the control groups. This is also important to prevent using too strong a stimulus that might overwhelm effects of running on seizure threshold. Based on this response curve, the remainder of the trials used a 42 mg/kg concentration of PTZ.

Conclusions

Physical exercise increased the seizure threshold in running mice as opposed to control mice. The last few trials were done using 42 mg/kg PTZ dose and no wheel for the non-running control groups (**S5-S6)**. The final results support my hypothesis. Animals that had access to running wheels exhibited lower seizure scores, with the mode of their seizure severity scores being lower than animals in the control group. This may be due to increased proliferation of DG neurons in running animals, and thus increased COX-2 expression. However, this remains to be examined.

Implications & Future Directions

Subjects allowed voluntary exercise were less susceptible to seizures, showing that running raised the seizure threshold and made it more difficult for exercised mice to experience severe seizures. I hypothesized that this is due to an increase in COX-2 levels specifically in the newly generated neurons that arose from the physical exercise. The brain slides that are stained with COX-2 must also be stained for BrdU. If it is found that the BrdU labeled neurons—the newly generated, exercised-induced neurons—co-localize with COX-2 labeled neurons, this will demonstrate that it is in fact these new neurons that produced higher levels of COX-2 in exercised mice, thus suggesting that this may play a role in the effects of exercise-induced neurogenesis on seizure activity. These studies have implications for future epileptic patients, in that treatment involving exercise—or more specifically running—may be used to perhaps decrease the frequency and severity of seizures in people with epilepsy. The noninvasive, cost-free, and simple treatment of exercise is beneficial in almost every aspect of life. These studies add to the evidence that voluntary exercise is neuroprotective and could not only increase performance in hippocampal functions, such as synaptic plasticity, learning, and memory, but also increase the seizure threshold.

Works Cited

1. Reynolds, E. A. (2005) Epilepsy: The Disorder. Epilepsy Atlas. Pamphlet. *World Health Organization* 16-19

2. Karis, J. P. (2008) Epilepsy. *American Journal of Neuroradiology* 28:1222-1224

3. NINDS. (2015) The Epilepsies and Seizures: Hope Through Research. *National Institute of Neurological Disorder and Stroke*<http://www.ninds.nih.gov/disorders/epilepsy/detail_epilepsy.htm>

4. Rojas, A., et al. (2014) Cyclooxygenase-2 in Epilepsy. *Epilepsia* 55(1):17-25

5. Turrin, N. P., Rivest, S. (2004) Innate IMmune Reaction in Response to Seizures: Implications for the Neuropathology Associated with Epilepsy. *Neurobiology of Disease* 16(2):321-334

6. Vezzani, A., Granata, T. (2005) Brain Inflammation in Epilepsy: Experimental and Clinical Evidence. *Epilepsia* 46:1724-1743

7. Claycomb, R. J., Hewett, S.J., Hewettt, J.A. (2011) Prophylactic, Prandial Rofecoxib Treatment Lacks Efficacy Against Acute PTZ-Induced Seizure Generation and Kindling Acquision. *Epilepsia* 52:273-283

8. Deng, W., Aimone, J.B., Gage, F. H. (2010) New Neurons and New Memories: How does Adult Hippocampal Neurogenesis Affect Leaning and Memory? *National Review Neuroscience* 11:339-350

9. Gobeske, K. T., et al. (2009) BMP Signaling Mediates Effects of Exercise on Hippocampal Neurogenesis and Cognition in Mice. *PLoS ONE* 4(10): e7506

10. Pragg, H.V., Kempermann, G., Gage, F. H. (1999) Running Increases Cell Proliferation and Neurogenesis in the Adult Mouse Dentate Gyrus. *Nature Neuroscience* 2(3):266-270

11. Praag, H. V. (2008) Neurogenesis and Exercise: Past and Future Directions. *Neuromolecular Medicine* 10:128-140

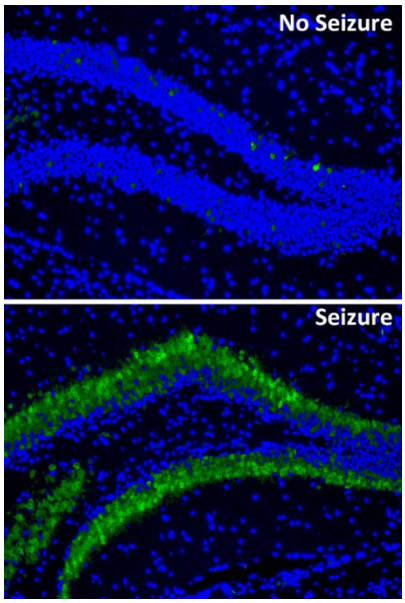
12. Praag, H.V. (1999) Running Enhances Neurogenesis, Learning, and Long-Term Potentiation in Mice. *Proceedings of the National Academy of Sciences* 96(20):13427-13431

13. The Jackson Laboratory (2014) Advantages of the Mouse as a Model Organism. <http://research.jax.org/mousegenetics/advantages/advantages-of-mouse.html>

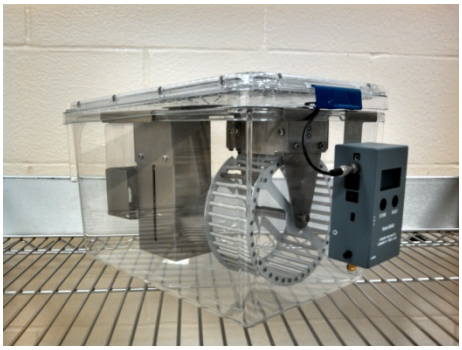
14. Bednarczyk, M. R., et al. (2011) Distinct Stages of Adult Hippocampal Neurogenesis are Regulated by Running and the Running Environment. *Hippocampus* 21:1334-1347

15. Tashiro, A., Makino, H., Gage, F.H. (2007) Experience-Specific Functional Modification of the Dentate Gyrus through Adult Neurogenesis: A Critical Period during and Immature Stage. *The Journal of Neuroscience* 27(12):3252-3259

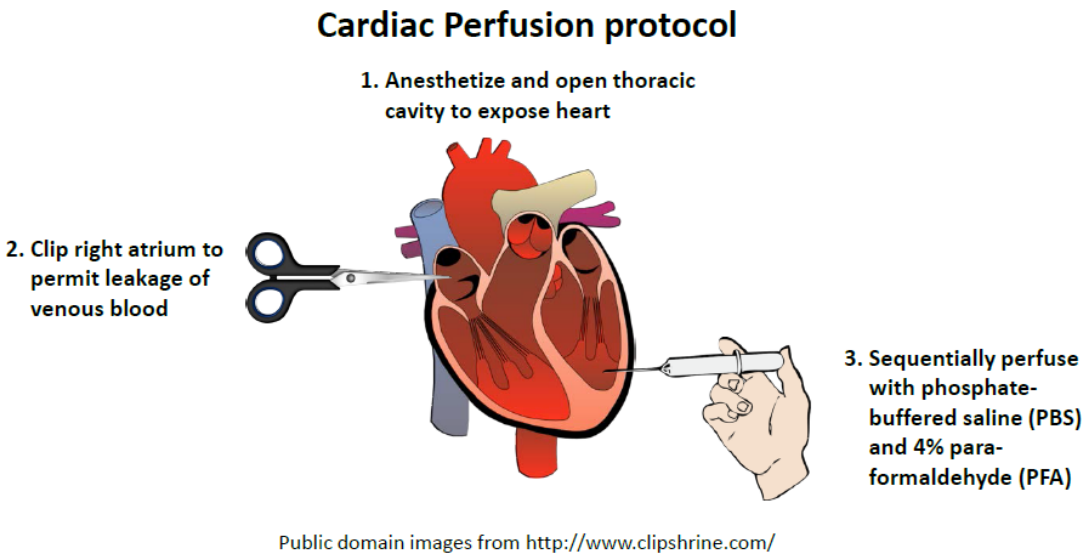
Appendix



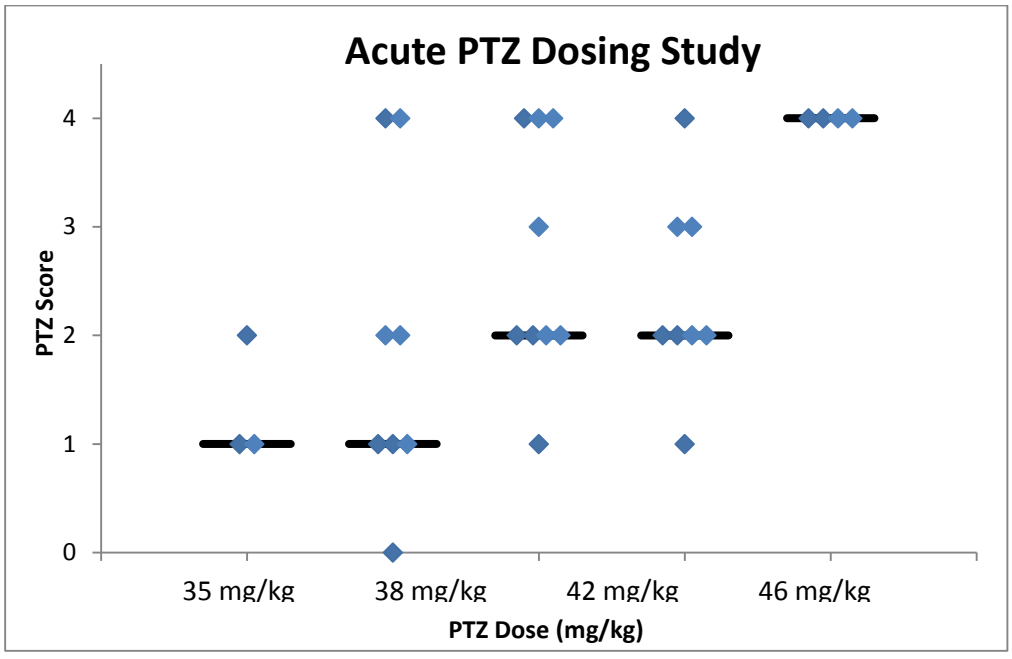
Supplementary 1. Increased COX-2 Expression in the Dentate Gyrus Three Hours after an acute Seizure. The figure shows two DGs, one from a mouse that had a convulsive seizure (lower panel) and the other that did not (upper panel). The green represents the COX-2 labeled neurons. (Taken with the permission of Yifan Gong)



Supplementary 2. Activity Wheel Cage and Manual Counter



Supplementary 3. Diagram of Cardiac Perfusion.



Supplementary 4. PTZ Dosing Response Curve. (Taken with the permission of Miriam Gladstone).