The impact of ketogenic diet on cerebral excitability

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THE IMPACT OF KETOGENIC DIET ON CEREBRAL EXCITABILITY

by

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I would like to dedicate this work to my parents for their unconditional support and love.
I look up to and love you both more than I can express in words. You guys are amazing!
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THE IMPACT OF KETOGENIC DIET ON CEREBRAL EXCITABILITY

IAN BENJAMIN

ABSTRACT

Many neurological disorders are a result of widespread changes in the excitability of brain tissue. The specific changes in neuronal excitability produces or worsens many of the symptoms associated with these disorders. Pharmacological methods are effective, but their associated side-effects are substantial, and often approximate the severity of the symptoms of the original disorder. The ketogenic diet, a high-fat, low-carbohydrate diet, has been shown to improve many neurological diseases by reducing hyperexcitability without the aforementioned side effects. The current study tested the hypothesis that a ketogenic diet would be associated with alterations in cerebral excitability.

Animals were fed either a control or ketogenic diet for at least 21 days prior to experimentation. Power spectral analysis was conducted using EEG data across frequency bands, and compared between diet groups. Current source density analysis was also performed to visualize potential alterations in cerebral excitability. In the second part of the experiment, a non-invasive ischemic stroke was delivered, and the excitability of the contralateral cortex was monitored.

No significant differences were observed between ketogenic and control experiments in regards to overall excitability, although ketogenic diet experiments showed a
significantly higher number of acute EEG depressions. No cortical spreading depression events were observed in contralateral recordings. Our findings are in contrast with data showing that ketogenic diets change overall basal excitability, but are in concert with other studies that show that ketogenic diets may not be associated with changes in excitability, but in changes in neuroplasticity.
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<tr>
<td>AcAc</td>
<td>Acetoacetate</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention Deficit-Hyperactivity Disorder</td>
</tr>
<tr>
<td>B-OHB</td>
<td>Beta-Hydroxybutyrate</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotropic Factor</td>
</tr>
<tr>
<td>Ca++</td>
<td>Calcium</td>
</tr>
<tr>
<td>CoSD</td>
<td>Cortical Spreading Depression</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>K&lt;sub&gt;atp&lt;/sub&gt;</td>
<td>ATP-gated Potassium Channel</td>
</tr>
<tr>
<td>KD</td>
<td>Ketogenic Diet</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Potassium</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-Term Potentiation</td>
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<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Sodium</td>
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INTRODUCTION

Modulating Brain Activity

Neurological diseases negatively affect activities of daily living by impairing brain activity and thereby producing functional deficits. Many neurological disorders are a result of widespread changes in the excitability of brain tissue. For example, epilepsy is a disorder that lowers neuronal thresholds for excitation, which manifests in seizures (Badawy, Vogrin, Lai, & Cook, 2013). Parkinson’s disease is characterized by an overall decrease in cerebral excitability, which manifests in ataxia, initiation tremors, and memory impairment (Suzuki, 2014). Changes in cerebral activity are also seen in stroke, Attention Deficit-Hyperactivity Disorder (ADHD), Alzheimer’s, depression, and even eating disorders such as anorexia (Khedr, El Fetoh, El Bieh, Ali, & Karim, 2014). The specific changes in neuronal excitability produces or worsens many of the symptoms associated with these disorders. Pharmacological agents are most frequently used to treat or control the symptoms of these disorders, although brain stimulation techniques have recently gained attention in the medical community (Stypulkowski, Stanslaski, Jensen, Denison, & Giftakis, 2014). While both of these methods are effective, their associated side-effects are substantial, and oftentimes can approximate the severity of the symptoms of the original disorder. Phenobarbital, which is one of the most widely used pharmaceuticals for epilepsy, often produces sleepiness, dizziness, memory problems, and depression in patients (Elafros, Bui, & Birbeck, 2014). Pharmaceutical compounds
are often prohibitively expensive, and may not be the preferred means of treatment by patients (Linsky, Simon, & Bokhour, 2015).

Another way by which brain activity can be controlled is through an alteration in diet. This approach consistently produces few to no side-effects, and has been shown to be beneficial in producing lasting alterations in cerebral excitability (Masino, Kawamura Jr., Ruskin, Geiger, & Boison, 2012). Diet-manipulation is also more cost-effective, and culturally flexible.

The goal of this thesis is to test the effect of a specific diet on cerebral excitability. To understand how brain activity changes in a disease state, it is necessary to understand basic brain metabolism. From there, the introduction will discuss how changes in nutrition can produce changes in excitability, and promote recovery in neurological disorders.

**Brain as a metabolic organ**

The brain is unique as it represents only 2% of total body mass, but accounts for 20-25% of the total energy consumption (Bélanger, Allaman, & Magistretti, 2011). It is the most energetically expensive organ in the body per unit weight, and preferentially uses glucose to fuel its cellular processes as opposed to fat. Even though the brain requires a constant source of energy, it does not convert stores of fat, glycogen or protein into glucose. The brain has the ability to sense and regulate blood glucose levels, with regions in the hypothalamic paraventricular and arcuate nuclei, and the nucleus tractus solitarius in the brain stem most integral for this function (Ogunnowo-Bada, Heeley, Brochard, & Evans, 2014). Such sensing is thought to be metabolic and non-metabolic in
nature. Metabolic sensing implies that the entry and metabolism of glucose in a neuron serves as the signal. Non-metabolic signaling could be mediated by activity of ATP-gated potassium channels (K\text{atp}), or linked to the activity of active glucose transporters in astrocytes and neurons (Ogunnowo-Bada, Heeley, Brochard, & Evans, 2014). K\text{atp} channels are also widely implicated in serving as a link between glucoregulation and neuroprotection (McCrimmon et al., 2005), and will be discussed later in this paper.

As seen in figure 1, glucose normally enters the neuronal cell through a GLUT-2 transporter and undergoes glycolysis in the cytoplasm to produce pyruvate. Pyruvate is transported into the mitochondria where it is converted into acetyl-CoA. Acetyl-CoA enters the citric acid cycle, producing intermediates to be used for oxidative phosphorylation. The end result is the production of ATP, which is the primary chemical energy source of the cell.

In periods of prolonged hypoglycemia, the brain uses intermediates of fat metabolism for energy. As triglyceride stores are larger than glycogen stores, and fat provides more kcal of energy per gram than does carbohydrate, this alternate method of energy production is very efficient. These metabolites also have the benefit of entering the mitochondria directly as shown in figure 1. Although this alternate form of metabolism is more efficient, cerebral tissue strongly prefers glucose for its metabolic demands (Harvey & Ferrier, 2011).
Figure 1: Visualization of the two energetic pathways in a neuron. A neuron that relies upon glucose metabolism must convert glucose into pyruvate. Pyruvate will be transported into the mitochondria, and enter the citric acid (TCA) cycle for ATP production. Glucose enters the cell through a GLUT-2 transporter. Ketone bodies enter the neuron through monocarboxylic acid transporters (MCT) which will be discussed later in the introduction. Once inside the neuron, the ketone bodies can directly enter the mitochondria for metabolism. Figure taken from (Lutas & Yellen, 2013).
Magnetic resonance spectroscopy utilizes radiolabeled carbon to visualize glucose oxidation and the rate of glutamate and GABA recycling in neurons and astrocytes. Using this technique, it has been estimated that 80% of brain energy demands are related to processes that support neuronal firing and cycling of neurotransmitters (Shulman, Rothman, Behar, & Hyder, 2004). The specific processes are the establishment and maintenance of ion-gradients through ATP-coupled ion pumps, as well as the manufacturing and recycling of neurotransmitters (Alle et al., 2009). Diets that more effectively funnel substrates into the citric acid cycle could improve the efficiency of these processes, especially if those diets improve energetics in periods of hypoxia. High-fat, low-carbohydrate diets are thought to do just that, an example of which is the ketogenic diet.

**Ketones and the Ketogenic Diet**

A ketone is an organic compound with the chemical formula \( RC(=O)R' \), where the carbonyl carbon is directly attached to a carbon molecule in \( R \) and \( R' \). A ketone is generally acidic with a pKa of approximately 4, which is why ketone bodies are also called keto-acids.

As a general guideline, a traditional ketogenic diet (KD) has a fat to carb/protein ratio of 4:1. This ratio is not strictly defined, and can be modified to whatever keeps ketone body concentrations sufficiently elevated. Protein intake recommendations are flexible, but normally account for 10-30% of calories (Lutas & Yellen, 2013). The major energy source of ketogenic diets are metabolites called ketone bodies, are produced by
the liver, and come in one of three water-soluble forms. They are acetone, acetoacetate (AcAc), and beta-hydroxybutyrate (β-OHB). These metabolites are produced from fatty acid oxidation. Free fatty acids (FFA) broken down from triglyceride in adipose tissue are transformed into the ketone bodies AcAc and β-OHB in the liver by the enzymes HMG-CoA lyase and β-OHB dehydrogenase, respectively (Harvey & Ferrier, 2011). The conversion of acetyl-CoA obtained from fatty acid oxidation into ketone bodies is accomplished entirely in the mitochondria, and is a process known as ketogenesis. Ketone bodies are then transported to extra-hepatic tissues with the necessary enzymes for reconversion into Acetyl-CoA. Acetyl-CoA can then enter the mitochondria to complete the TCA cycle as usual. This process is depicted below.

**Figure 2. Endogenous Ketogenic Pathway.** FFAs broken down from TG in adipose tissue are transformed into the ketone bodies β-OHB and AcAc in the liver. Subsequently, these substances are transported to extra-hepatic tissues in possession of the necessary enzymes for reconversion into Acetyl-CoA. Acetyl-CoA can then enter the mitochondria to complete the TCA cycle as usual. Figure taken from (Harvey & Ferrier, 2011).
When ketone bodies are chronically elevated and glucose is depleted, the body uses them as energy substrates in a state known as ketosis.

For a tissue to successfully utilize ketone bodies for energy, it must be able to convert ketone bodies to acetyl-CoA. Thiophorase, or succinyl-CoA:acetoacetate-CoA tranferase is the enzyme that determines this. It reconverts the products of fat breakdown, acetoacetate (AcAc) and succinyl-CoA, into the products acetoacetyl-CoA and succinate. Thiolase subsequently converts acetoacetyl-CoA into acetyl-CoA for entry into the TCA cycle, which, as mentioned earlier provides substrates necessary for energy production. Because the liver lacks thiophorase, it cannot use ketone bodies as fuel substrates. Since the brain does possess thiophorase, it gains the metabolic flexibility to use ketone bodies as energy sources during hypoglycemia.

Ketosis typically occurs during prolonged periods of starvation, beginning around 24 days without food as seen in figure 3.

**Figure 3. Metabolic changes induced during starvation.** Figure taken from (Cahill, 2006).
This process can also be achieved amidst normal caloric intake by maintaining a very high fat and very low carbohydrate intake (Cahill, 2006). Ketone body concentration will reach sufficient concentrations for metabolism at approximately 3 weeks of starvation, or 3 weeks of a proper KD.

Fat intake needs to be sufficiently high to provide enough substrate for ketogenesis, and glucose intake needs to be kept low enough to suppress insulin release. Insulin is released with increases in blood glucose concentrations, helps shuttle blood glucose into cells for metabolism, and potently inhibits fatty acid oxidation (Harvey & Ferrier, 2011). This leads to a reduction of ketogenic metabolism. Lastly, protein consumption needs to be monitored, as proteins can be converted into glucose and trigger insulin release. For example, certain amino acids such as leucine can trigger potent spikes in insulin levels (Harvey & Ferrier, 2011).

There is some concern that KD could lead to acidification of the blood due to the changes seen in uncontrolled diabetes. In diabetes, the body is non-responsive to the hormone insulin (Harvey & Ferrier, 2011). Because insulin cannot effectively shuttle glucose into cells, the body reacts as though it was starving. The result is an elevation in fatty acid breakdown, and elevation of ketone body concentration in the blood. These keto-acids account for the acidification of blood in uncontrolled diabetes. As depicted on the following page in table 1, studies investigating ketogenic acidosis found no changes in blood pH of healthy subjects, suggesting that these diets should not be a concern for patients with disorders of the brain only (Paoli, Rubini, Volek, & Grimaldi, 2013).
Table 1: Blood pH levels associated with normal, high-carbohydrate diets vs controlled ketogenic diets vs diabetic ketoacidosis. pH levels are unsignificantly different between healthy subjects regardless of diet. Table taken from Paoli, Rubini, Volek, & Grimaldi, 2013.

<table>
<thead>
<tr>
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<th>Normal diet</th>
<th>Ketogenic diet</th>
<th>Diabetic ketoacidosis</th>
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<tr>
<td>Glucose (mg/dl)</td>
<td>80–120</td>
<td>65–80</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>Insulin (µU/l)</td>
<td>6–23</td>
<td>6.6–9.4</td>
<td>≈ 0</td>
</tr>
<tr>
<td>KB conc (mM/l)</td>
<td>0.1</td>
<td>7/8</td>
<td>&gt; 25</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.4</td>
<td>&lt; 7.3</td>
</tr>
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In addition to possessing the correct enzymes necessary for reconverting ketone bodies into acetyl-CoA, a tissue must first absorb the ketone bodies from the blood stream. For most organs this is not a problem, as the blood supply is not filtered prior to arriving at the tissue. But the brain is unique in that is has a protective barrier between the blood and the CSF, known as the blood brain barrier (BBB).
Figure 4. Cross section of a brain microvessel. Endothelial cells are ensheathed by the basal lamina which contains pericytes. Astrocytic endfeet are connected to the basal lamina, and are in contact with glia and neuronal brain tissue. Figure taken from (Mahringer, Ott, & Fricker, 2013)

Shown in figure 4, the BBB is a highly selective, semi-permeable barrier that separates blood from brain tissue. For the brain tissue to utilize a given substance, the compound must pass through the BBB. Ketones are too polar and too large to diffuse across the BBB, and require the action of specialized transporters known as monocarboxylic-acid transporters (MCT).

There are two types of these transporters, MCT1 and MCT2, both of which are present in vascular and non-vascular tissue, and in neuronal and non-neuronal tissue (Prins, 2008). These transporters are responsible for the transport of ketone bodies into all tissues. A neuronal MCT transporter can be seen in figure 1. Not pictured are MCTs on
astrocytes and endothelial cells. Under conditions of high glucose intake, the expression of these two transporters in astrocytes, endothelial cells, and neurons is relatively constant. If a KD is employed, as shown by Moore in 1976, the expression and activity of these transporters changes depending upon concentration and the identity of fuel substrates present, respectively. As a result, even if the concentration of ketone bodies does not change over the course of 1 week, the activity of MCT1 and MCT2 will still increase due to the activating presence of ketones themselves. Increased MCT1 and MCT2 expression results in better ketone body uptake, and more substrate availability for metabolism as shown in table 2. In a state of ketosis, brain ketone metabolism is limited by the cerebral uptake of ketone bodies, not by the activity of thiolase or thiophorase (Prins 2008).

**Table 2. MCT Levels following CNS injury.** Figure taken from (Prins, 2008)

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<th>Time course</th>
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<td>Traumatic brain injury</td>
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<td>6, 24 h</td>
<td>PND35, -90 rat</td>
<td>Increased MCT2 expression in microvessels</td>
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<tr>
<td>Ischemia</td>
<td>MCT1</td>
<td>3, 21 days</td>
<td>Adult rat</td>
<td>Increased MCT1 in astrocytes and endothelia</td>
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<td></td>
<td>MCT1, -2, -4</td>
<td>6 to 120 h</td>
<td>Adult rat</td>
<td>Increased MCT1, -2, -4 mRNA in infract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 to 24 h</td>
<td></td>
<td>Increased MCT1 protein</td>
</tr>
<tr>
<td>Hemorrhagic shock</td>
<td>MCT1</td>
<td>2 h</td>
<td>340 to 388 g rat</td>
<td>Increased MCT1 expression in animals resuscitated with β-OHB</td>
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<td>Hypoxia</td>
<td>MCT1</td>
<td>1 day</td>
<td>Cultured astrocytes</td>
<td>Increased MCT1 expression 1 day after hypoxia</td>
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<td>MCT1 protein</td>
<td>4 to 6 weeks</td>
<td>150 to 190 g rat</td>
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<td>Noradrenaline</td>
<td>MCT1 and MCT2</td>
<td>6 h</td>
<td>Cultured neurons</td>
<td>Within 2 h after noradrenaline application MCT2 expression increased</td>
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CNS, central nervous system; MCT, monocarboxylate transporter.
Metabolic Changes of Stroke

Ketogenic diets have been shown to reduce hyperexcitability in the brain, gaining importance in the context of neurological diseases like stroke, in which excitability is heightened.

A neuronal ischemic event occurs when a blood clot from the peripheral or central blood vessels lodges and occludes blood flow in a cerebral artery. The result is a stereotypic cascade characterized by a drop in ATP stores, a loss of ionic homeostasis, intracellular lactate accumulation, and extracellular glutamate accumulation (Iadecola & Anrather, 2011). It is hypothesized that the Na\(^+\)/K\(^+\) ATPase pump malfunctions due to the loss of ATP, leading to an accumulation of K\(^+\) outside of the neuron, thereby triggering Na\(^+\) and Ca\(^{++}\) cytoplasmic influx. This influx leads to a massive release of excitatory glutamate and prolonged stimulation of AMPA and NMDA ionotropic receptors (Moskowitz, Lo, & Iadecola, 2010). The stimulation of these receptors triggers further Ca\(^+\), Na\(^+\), and water influx into neurons. Furthermore, glutamate recycling is impeded due to the loss of ATP, as illustrated in figure 5 on the following page.
Figure 5: Astrocyte mediated glutamate recycling. Following glutamate release, recycling of the neurotransmitter is accomplished with the assistance of neighboring astrocytes. These astrocytes utilize the electrochemical gradient of Na⁺ maintained by the Na/K ATPase as a driving force, and glutamine synthetase to interconvert glutamate to glutamine. Thus, Na uptake and glutamate recycling are tightly coupled. Additionally, astrocytic glutamate is converted into glutamine by glutamine synthetase, released into the synaptic cleft, reuptaken by the neuron, and reconverted into glutamate for future release. This conversion also requires energy derived from ATP. This presents a problem seen in cerebral ischemia, in which oxygen levels drop and ATP production cessates. In this scenario, insufficient ATP is produced to power either the Na/K ATPase pump or glutamine synthetase, and as a result, the Na gradient is disrupted and glutamate accumulates in the cleft. Figure taken from (Magistretti, Pellerin, Rothman, & Shulman, 1999).
The accumulation of intracellular Ca\(^{++}\) triggers calcium-dependent proteases such as caspases to produce free radicals, vasodilatory NO, and arachidonic acid metabolites. If the cascade is not interrupted, caspase-dependent apoptosis is initiated, resulting in cell death and necrosis.

**KD relevance to excitability**

All of these aforementioned cellular changes should amount to elevated brain activity. The idea behind KD is that they provide an alternative means for energy production in an anaerobic environment. By maintaining ion-flux and glycolytic stability amidst cerebral injury, the KD should antagonize changes in neuronal membrane potentials, and thus, mitigate changes in cerebral excitability.

Ketogenic diets have not been shown to alter homeostatic concentrations of intracellular and extracellular ions (Hartman, Gasior, Vining, & Rogawski, 2007), but they have been postulated to change ion channel permeability. For example, \(\beta\)-OHB administration reduced Ca\(^{++}\) entry following neuronal NMDA activation (Lund, Ploug, Iversen, Jensen, & Jansen-Olesen, 2015). Given that intravenous \(\beta\)-OHB administration after an ischemic event reduced cerebral infarct volume by 50% (Prins, 2008), the KD alteration of Ca\(^{++}\) influx is of interest.

Recent studies support that Ca\(^{++}\) influx can occur via glutamate independent acid-sensing ion channels (ASICs). These channels are sensitive to intracellular acidification seen during anaerobic glycolysis, and have been shown to trigger intracellular calcium
flux independently of glutamate (Simon & Xiong, 2006). Not only does anaerobic glycolysis produce acid, it produces lactate. It has recently been shown that lactate is one of the most important enhancers of ASIC function (Wang & Xu, 2011).

Similarly, ATP-sensitive K\(^+\) channels have been characterized in both neurons and glial cells, and serve as molecular sensors of cellular metabolism (Nichols, 2006). K\(_{\text{atp}}\) channels are located on both the neurolemma and the mitochondrial membrane, and are thought to open when the ATP/ADP ratio is reduced. K\(^+\) efflux hyperpolarizes the cell, reducing the probability of cytosolic Ca\(^{++}\) influx from voltage-gated Ca\(^{++}\) channels (Akrouh, Halcomb, Nichols, & Sala-Rabanal, 2009). The relationship between KD and K\(_{\text{atp}}\) is paradoxical, as a boost in ATP from KD should inhibit these channels, not activate them as has been observed. To reconcile this, it has been shown that the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase directly regulates K\(_{\text{atp}}\) channel activity, suggesting that our understanding of K\(_{\text{atp}}\) permeability is incomplete (Lund, Ploug, Iversen, Jensen, & Jansen-Olesen, 2015). High ATP levels obtained through a ketogenic diet should suppress glycolytic flux, as ATP and the citric acid cycle intermediate citrate are feedback inhibitors of glycolysis. It is also hypothesized that polyunsaturated fatty acids obtained through the KD may also activate K\(_{\text{atp}}\) channels, albeit indirectly (McCrimmon et al., 2005). It was shown that fatty acids intercalated in neuronal membranes strongly interacted with K\(_{\text{atp}}\) channels, and potently reduced their affinity for ATP (Shyng & Nichols, 1998). The apparent paradox between ketosis and activation of K\(_{\text{atp}}\) channels may be due to an incomplete understanding of the channel’s kinetics, and warrants further investigation.
While the glucose-dependent brain increases anaerobic glycolysis in the face of hypoxia, the ketogenic brain is able to significantly bypass glycolysis. To support this, the administration of ß-OHB reduces neuronal glycolytic rate in normoxic conditions (Lund, Ploug, Iversen, Jensen, & Jansen-Olesen, 2015). If the aforementioned hypotheses surrounding ASICs and K\textsubscript{atp} channels are true, ketone metabolism should boost K\textsubscript{atp} and reduce ASIC conductance, antagonizing hyperexcitability. Furthermore, the anaerobic glycolytic byproduct lactate increases extraneuronal prostaglandin E2 through the inhibition of prostaglandin transporters in astrocytes (Carmignoto & Gómez-Gonzalo, 2010). Prostaglandins are immunological vasodilators that also promote the formation of platelet-aggregating thromboxane A2, worsening the prospect of neuronal clot dissolution ("Stroke Prevention | Internet Stroke Center,” n.d.).

KDs are also thought to lower brain-derived neurotropic factor (BDNF) levels, either through elevation of poly-unsaturated levels of fatty acids, or through depression of glucose concentrations. Although BDNF is generally considered to be neuroprotective and stimulate neurogenesis, it has also been shown to be pro-epileptic. For example, developmental knockout of the BDNF receptor TrkB has eliminated epileptic symptoms altogether in certain models (McNamara & Scharfman, 2012). This could be BDNF’s role of potentiating glutamate receptor function by promoting phosphorylation of excitatory NMDA receptor type 1 on residue Ser-897 (Slack, Pezet, McMahon, Thompson, & Malcangio, 2004). This could also be because BDNF promotes expression of excitatory glutamate receptors type 1 and 2 (Caldeira et al., 2007). BDNF synthesis is partly regulated by levels of cytosolic NADH. Higher levels of glycolysis result in higher
levels of cytosolic NADH, higher BDNF, and a higher probability of seizure. Because KDs reduce glycolysis, they should reduce BDNF levels, and reduce excitability.

Even if the proposed mechanisms are incorrect, KDs have been shown to exhibit neuroprotective effects in vitro, in vivo, and in a few clinical studies. For example, the administration of AcAc infusion for 14 days to both in vitro and in vivo rodent models of glutamate-induced neurotoxicity resulted in decreased neuronal damage (32%), reduced lesion volume (50%), and improved cellular ATP (Massieu et al., 2003). Although relatively sparse, the existing clinical data suggests that KDs warrant future research for neurodegenerative conditions, neurotraumatic injuries (Davis et al., 2008), and any other condition in which brain metabolism may be altered. (Krikorian et. al, 2012). For example, one study used intravenous β-OHB to reduce severity of symptoms in patients diagnosed with Alzheimer’s disease by increasing paragraph recall accuracy relative to control subjects (Reger et al., 2004). However, clinical data also document physical complications such as elevated total cholesterol, LDL cholesterol, and TG levels, all of which need to be considered and weighed against neuroprotective benefits in a clinical setting (Güzel, Yılmaz, Uysal, & Arslan, 2015).
OBJECTIVES

The objective of this paper is to investigate a potential relationship between ketogenic diets and cerebral excitability. Specifically, we will:

1. Compare the electroencephalogram (EEG) from animals exposed to a ketogenic diet with those exposed to a control diet. EEG measurements will be made in the parietal cortex of anesthetized animals, and evaluated using power spectral density estimates.

2. Evaluate potential alterations in excitability after non-invasive induction of a stroke event.

Throughout this study, we will test the hypothesis that the presence of the ketogenic diet produces alterations in cerebral excitability.
METHODS

Subjects:

Male Sprague-Dewey rats, (n=20, 385-490g) were used in these experiments. Animals arrived and were given one week to acclimate to their new housing environment prior to implementation of their specific diets. The housing environment was temperature maintained between 70-74 degrees Fahrenheit with 12:12 hour light-dark cycle, lights turning on at 6 am and off at 6 pm. After the acclimation period, all rats were fasted for 18 hours to stabilize the concentrations of blood glucose, and initiate a state of ketogenesis (Puchowicz et al., 2008). All procedures were conducted in strict accordance with the Institutional Animal Care and Use Committee of Boston University’s approved guidelines.

Diet Manipulation

Rats were randomly and evenly assigned to either an ketogenic or irradiated ingredient-matched control diet group, both of which were fed ad libitum for a minimum of three weeks prior to experimentation. The ketogenic diet was formulated with a fat:protein+carbohydrate ratio of approximately 4.2 and provides 6.7kcal/gram, while the control diet has a fat:protein+carbohydrate ratio of approximately .06 and provides 3.6 kcal/gram. Both diets were irradiated prior to incorporation. The control diet provided 77% of calories from carbohydrate, with the caloric remainder being equally split between fat and protein, a ratio designed to mimic a classic Western diet. Both diets were
obtained from Envigo Teklad Diets, with formulation assistance from nutrition expert Dr. Tina Herfel. The macronutrient, micronutrient and ingredient source breakdowns are listed in table 3 below. The KD was fed by filling petri dishes with the feed, with care taken to place the dish above their bedding. Diet was replaced daily to avoid contamination of the feed with kicked-up bedding.

Rats were paired-housed. Because pair-housing may result in bullying particularly with dietary manipulation weights were monitored daily for disparities between cage partners. None were observed.

Table 3: Macronutrient and micronutrient breakdown of Envigo Teklad diets utilized.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TD.110870</th>
<th>TD.110870</th>
<th>TD.110871.PWD</th>
<th>TD.110871.PWD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
<td>g/kcal</td>
<td>g/kg</td>
<td>g/kcal</td>
</tr>
<tr>
<td>Casein</td>
<td>100.0</td>
<td>27</td>
<td>180.0</td>
<td>27</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>6.6</td>
<td>0.17</td>
<td>6.6</td>
<td>0.17</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>25.0</td>
<td>7</td>
<td>85.0</td>
<td>13</td>
</tr>
<tr>
<td>Cellulose</td>
<td>35.4</td>
<td>9.7</td>
<td>60.11</td>
<td>8.9</td>
</tr>
<tr>
<td>Vitamin Mix, AIN-93-VX w/ Cellulose</td>
<td>14.5</td>
<td>4.0</td>
<td>26.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2.5</td>
<td>0.7</td>
<td>4.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Mineral Mix, w/o Ca &amp; P (58057)</td>
<td>13.39</td>
<td>3.7</td>
<td>24.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Calcium Phosphate, dibasic</td>
<td>9.8</td>
<td>2.7</td>
<td>17.84</td>
<td>2.6</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>5.25</td>
<td>1.4</td>
<td>9.54</td>
<td>1.4</td>
</tr>
<tr>
<td>TBHQ, antioxidant</td>
<td>0.07</td>
<td>0.02</td>
<td>0.13</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Values are calculated from ingredient analysis or manufacturer data.
Animals were weighed immediately prior to experimentation, and subsequently anesthetized with sodium pentobarbital (40mg/kg) and chloral hydrate (160mg/kg). Glycopyrolate (0.02mg/kg) was injected to reduce respiratory secretions.

Once the animal was non-responsive to a tail pinch, tail vein catheterization was performed. Following successful catheterization, the animal was placed in a rodent stereotaxic apparatus (Kopf instruments, Tujunga, CA) to ensure immobility of the cranium. A midline incision was made, and skin and fascia were reflected to expose the frontal, parietal and occipital bones.

**Recording Electrode Placements:**

Twelve animals (6 control, 6 ketogenic) underwent electrophysiological monitoring of the hemisphere contralateral to the stroke. A hole in the skull was drilled 0.5mm anterior, and +3.5mm lateral of lambda to introduce a multicontact recording electrode into the brain. Drilling was performed with a saline wash to prevent heating of the bone. Two more drill sites were marked above and below the landmark bregma, halfway between midline and the right edge of the fascia. These became the sites of the indifferent and ground electrodes. An additional five animals underwent the same procedure, except the recording electrode was placed on the same side of the lesion.

A 23-channel recording electrode with 75-micron electrode spacing (Plexon) was used to simultaneously record brain signals from throughout the neocortical layers. The recording electrode apparatus was connected to the ground leads, and all were connected
to a headstage preamplifier that was connected to the main amplifier (Neurotrack systems; Ulbert et al., 2001).

**Focal Ischemic lesion:**

After placement of the recording electrode, a fiber optic light was placed flush and perpendicular to the skull. The light was placed on the left rostral parietal bone. Forty-five minutes after placement of the recording electrode, five 5-minute long baseline recordings were made. The data collected from the electrode was registered and saved on the computer using a custom-made program on National Instruments LabVIEW software. Thereafter, a 7-minute recording was initiated, during which all room lights were turned off, the fiber optic white light was turned on, and 0.5ml of Rose Bengal was slowly injected into the tail catheter with a 2ml flush of saline. The interaction of the light with the intravascular Rose Bengal produced a photothrombic stroke with associated cortical spreading depression, the latter of which could be visualized from the recorded EEG. Following the stroke event, 1 hour of recordings were conducted in 5-minute intervals for contralateral animals, and 2 hours of recording for ipsilateral animals.

Upon completion of the experiment, the rat was given a lethal dose of pentobarbital and intracardially perfused with approximately 500mL of 4% paraformaldehyde in 0.1M phosphate buffer, pH=7.4. Sodium nitrate and heparin were administered prior to the fixative. After the fixation, the brain was post-fixed in 4% paraformaldehyde.
Analysis:

The first area of focus for this study revolved around potential differences in neuronal excitability between KD and control rats. For this component of the study, contralateral recordings were used. To determine if there were differences in basal excitability, we evaluated frequency content by performing a multitaper spectrogram on the input voltage signals. For this analysis, the power spectral density for each channel was estimated during each file over specific frequency values and plotted. Multitaper estimates were performed using a time-bandwidth product of 3, and 5 tapers over a frequency range of 0 to 80Hz. The resulting power spectra vectors were then averaged across animals for the appropriate time and frequencies. Values were normalized and subsequently evaluated for statistical significance.

The second analysis utilized current source density analysis (CSD, Pettersen et al., 2007) of the coincident voltage signals from the recording electrode to evaluate the presence of a cortical spreading depression. The electrostatic forward solution was calculated using a spline smoothing to generate the current source density estimate. The number of waves and their timing was counted. This method of analysis allowed for spatiotemporal visualization of outward and inward current flow from an extracellular perspective, while factoring out currents not in the immediate vicinity of the recording electrode.
RESULTS

Table 4: Record of animal data for experiments. Contralateral measurement for Drill site: + 0.5mm from lambda, +3.5mm from lambda, measured using controller arm on the left sided stereotaxic placement. Ipsilateral measurements for drill site: + 0.5mm from lambda, +3.5mm from lambda, measured using controller arm on the right sided stereotaxic placement. Location of white optic light: just inferior and left to bregma.

<table>
<thead>
<tr>
<th>Date</th>
<th>Rat</th>
<th>Type</th>
<th>Weight (g)</th>
<th>Notes</th>
<th>Stroke time</th>
<th>Low frequency dips observed</th>
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</thead>
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<tr>
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<td>Control</td>
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<td>Contralateral</td>
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<td>385</td>
<td>Contralateral</td>
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</tr>
<tr>
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<td>Keto</td>
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<td>Contralateral</td>
<td>7min</td>
<td>10</td>
</tr>
<tr>
<td>2.7.16</td>
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<td>Keto</td>
<td>435</td>
<td>Contralateral</td>
<td>7min</td>
<td>7</td>
</tr>
<tr>
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<td>Keto</td>
<td>470</td>
<td>Contralateral</td>
<td>7min</td>
<td>5</td>
</tr>
<tr>
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<td>12</td>
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<td>460</td>
<td>Contralateral</td>
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<tr>
<td>2.22.16</td>
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<tr>
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<td>Ipsilateral</td>
<td>7min</td>
<td></td>
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<tr>
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<td>Control</td>
<td>490</td>
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<td>490</td>
<td>Ipsilateral</td>
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<td>440</td>
<td>Contralateral</td>
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</tr>
<tr>
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<td>465</td>
<td>Contralateral</td>
<td></td>
<td></td>
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<tr>
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<td>Keto</td>
<td>470</td>
<td>Contralateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.9.16</td>
<td>failed</td>
<td>Keto</td>
<td>460</td>
<td>Contralateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.12.16</td>
<td>failed</td>
<td>keto</td>
<td>460</td>
<td>Contralateral</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Animal Data

Animals were weighed immediately prior to experimentation. Mean weight for controls was 459.2g with a SD=30.5, and mean weight for ketogenic animals was 445.8g, with a SD=16.85. There was no statistically significant difference in mean weights between animals. Approximately 2 weeks into the feeding period, the KD animals developed porphyrin secretions on and around their eyes and noses. These secretions are associated with stress or allergies in rats. We hypothesized that due to the soft nature of their diet, they were unable to grind their teeth down or chew on anything, and were developing the secretions as a result. Irradiated wood blocks were placed into their cages to gnaw on, which seemed to improve symptoms.

Experimental consideration

During the experiments, each animal was sedated and anesthetized. As indicated by table 1, a disproportionate number of animals in the ketogenic group exhibited premature death from the anesthetic prior to experimentation. While it is clear that the ketogenic group was evaluated earlier than the control diet animals and this effect could be a result of practice and experience, it is also worth considering that the animals subjected to a ketogenic diet may have had a differential susceptibility to the anesthetic.
**Power analysis**

We hypothesized that ketogenic diets would alter basal neuronal excitability metrics compared to controls. To evaluate this hypothesis, averages and standard errors for power spectra were calculated and averaged for each epoch across frequency values. Results are depicted in figure 6.

**Figure 6: Power averages across recordings across frequencies.** Frequencies bands were split into two groups to better visualize the power for alpha, beta and gamma bands.
There was high variance in measures between animals, and t-tests revealed no significant differences in any of the comparisons. P values for t-tests of raw averages of delta, theta, alpha, beta gamma, and total power values were .506, .331, .307, .309, .538, and .831 respectively. To reduce standard error, data were normalized, and then evaluated again with t-tests. P values for normalized delta, theta, alpha, beta and gamma frequencies were .338, .520, .537, .427, and .830 respectively. A visual representation of the normalized power averages across frequency bands is shown in figure 6.

This suggests that there are no differences in basal excitability metrics between ketogenic and control-diet fed animals. These estimates of EEG power were generated for 5-7 minute blocks.

Excitability was also examined at a finer time course by generating and evaluating spectrograms. These spectrograms were characterized by a high power at low frequencies, which became lower at higher frequencies. In both groups, spectrograms revealed temporary reductions in power at low frequencies (0-30Hz). These events were referred to as dips. Dips were present in delta (0.1-3Hz), theta (4-7Hz), alpha (8-15Hz), and beta (16-31Hz) ranges. An example of the dip is pictured in figure 7 at epochs 24 and 27. Dips were counted and totaled for all KD and control contralateral animals, and were statistically evaluated using chi-square analysis. Two different chi-squares were conducted, one with expected dip occurrence values equal to those observed for control experiments, and another with expected values equal to the average between control and ketogenic experiments. The chi-square for both conditions were statistically significant,
the first with a p<.05 (p=.001) for 1 degree of freedom, and the second with a p<.05 (p=.008)

The primary EEG recording data was used to assess what these low frequency dips represented. As shown in figure 7 on the following page, the dip on the spectrogram corresponded to a period of EEG depression. The compressed EEG data shows an absence of any precipitating event for this depression. This suggests that ketogenic diets produced more acute periods of hypoactivity than did controls, but the basis for this is unclear.
Figure 7: Low frequency dip data. Primary EEG data (bottom) that corresponds to the low frequency dip visualized in the power spectra (top). Depressed EEG activity was consistently seen during dip time intervals.
Current source density analysis

Current source density analysis was performed on EEG recordings for contralateral and ipsilateral animals. Plots were made, which allowed for the spatiotemporal visualization of ion flow in the cortex following induction of stroke.

In one animal, recordings were made ipsilateral to the stroke. Two CoSDs within this animal were visualized using current source density, one of which is pictured below in figure 8. The events occurred approximately 33 and 80 minutes after the stroke was induced. There were no CoSDs were visualized in any of the contralateral current source density plots.

Figure 8: CSD plot showing CoSD in ipsilateral pilot approximately 33 minutes after induction of stroke ended. Blue represents a current source, and red indicates a current sink.
Frequency analysis was conducted at the same time points for the successful ipsilateral experiment. As observed below, low frequency amplitude was reduced prior to the onset of CoSD on the EEG by a margin of approximately 100 seconds. This served as a standard for comparison against other power spectra. No power spectra for other animals showed similar changes in low-frequency amplitudes over time.

A.  

B.  

**Figure 9: Ipsilateral CoSD data.** On top, a CoSD event is visualized in EEG data. On bottom, the same CoSD event is boxed. There was a smooth depression in low-frequency power (red) approximately 100 seconds prior to the CoSD event.
DISCUSSION

Results

This study found no alteration in excitability in the ketogenic diet group relative to the control group. While an increase in the number of ‘dips’ in the ketogenic group was observed, they were not due to transient spreading depression events. Because of this, the basis of this difference is unclear. As a positive control for cortical spreading depression measurements, a recording ipsilateral to the site of stroke was conducted. This experiment measured two cortical spreading depression events.

Stroke, Cortical Spreading Depression, and Hyperexcitability

There are several metrics that were used to assess for diet-induced changes in cerebral excitability. The first involved power spectral density analysis to evaluate baseline levels of cerebral excitability. Another involved monitoring cerebral activity contralateral to a stroke. During a stroke, it is believed that the contralateral hemisphere becomes more excitable for up to 1 week (Johansson, 2000). Also coincident with stroke are phenomena known as Cortical Spreading Depressions (CoSDs) and peri-infarct depolarizations (PIDs). CoSD immediately follows brain injury, ischemic stroke, subarachnoid and intracranial hemorrhage, and is believed to be associated with the aura of migraines (Lauritzen et al., 2011). Because these phenomena alter cerebral excitability, it was also of interest to test if the low frequency dips observed in the spectrograms were
associated with, or were precipitated by CoSD or PIDs that spread to the contralateral hemisphere.

CoSD occurs due to failure of ion gradient maintenance in the brain. This failure propagates as a wave through cortical gray matter at a rate of 2-5mm/min, transiently interrupting function of the cortex (Lauritzen et al., 2011). In the wake of CoSD, neurons become hyperpolarized and hypoexcitable for a prolonged period of time, which is visually confirmed through depressed EEG activity. Glucose consumption, glutamate transmission, and ion flux is increased during CoSDs, with profound extracellular K+ accumulation (Enger et al., 2015). Peri-infarct depolarizations are waves that induce the same effects as CoSD, but which are caused specifically from ischemic events (Fabricius et al., 2006). These waves begin in tissue directly adjacent to the ischemic core, or the penumbra. Recurrent PIDs in the injured brain are associated with increased cell death (Busch, Gyngell, Eis, HoehnBerlage, & Hoosmann, 1996). The reestablishment of ion-gradients is energetically expensive as mentioned previously. Without oxygen and adequate perfusion, PIDs increase in frequency, and tissue damage worsens (Fabricius et al., 2006).

Limitations:

There are several limitations with this study. A major limitation is the small sample size, which hampers statistical power and the validity of our results. Secondly, the study was conducted in rats. Although many similarities between rat and human brain structure and function exist, there are important differences that limit the conclusions of
metabolic studies of this nature. It has been shown that MCT-1 and MCT-2 density decreases over time for rats, and that even for young rats, the brain dependence on ketone bodies is relatively less than it is for humans (Prins, 2008). It is unclear if ketogenic metabolism would be as beneficial in rats as it would be in humans, and more research needs to be done on this. Clinical data are sparse for ketogenic diets, although some studies do show promising results for patients with Alzheimer’s and intractable epilepsy.

Another consideration is that oxygen is still required for the electron transport chain. Even if KDs do funnel metabolic substrates into the citric acid cycle more efficiently than glucose metabolism, without oxygen, electron acceptor recycling necessary for the TCA cycle and ETC will be impaired. Because of this, the conclusions we draw for KD may not apply to all neurological diseases, and should be considered mainly in light of stroke.

**Confounds:**

There are several confounds within this experiment. Un-blinded execution and analysis of experiments may have accounted for a large source of bias. Because KD experiments were completed prior to the control experiments, the experimenter likely became more proficient with experimental techniques over time. This may mean that later recordings were more reliable.

Food rations were intended to be ad-libitum, but rationing of the KD had to be utilized 3 weeks after feeding started due to limited supplies. As the KD had a frosting-like consistency, care was taken to place the diet-filled petri dishes above the animal’s
bedding. Still, animals would kick bedding into the petri dishes, which made accurate monitoring of accessible food difficult. Animal weights were taken daily to assess for disparities, and to ensure that KD animals were gaining weight at a similar rate to controls. Although age did differ between ketogenic and control animals by approximately 12-24 days at the onset of experimentation, there is no precedent for believing that this would have a significant effect upon ketogenic metabolism.

**Improvements:**

In regards to the experimental methods, some improvements could be made. Staggered feeding could have been utilized from the beginning of the study. Staggering the feeding start times would provide more consistency between animals for time spent on a diet, improve strength of conclusions surrounding metabolic changes, and preserve diet stores.

Ipsilateral recordings for rats 157 through 160 were conducted with a damaged recording electrode, which likely accounts for the absence of observed CoSD during analysis of these animals.

**Conclusions:**

Analysis of power spectra averages across animals showed no differences in basal excitability between the two groups. Variability was high between and within recording and no significance could be attributed to differences in average power data between
animal groups. When the measures were normalized to total power, there were still no detected differences. This finding supports several other studies that also show no changes in basal excitability in rat models (Blaise, Ruskin, Koranda, & Masino, 2015).

However, there was a statistically significant difference (p<.01) in the number of low-frequency dips amongst contralateral recordings. As noted in the results portion of this paper, the dips in frequency were associated with periods of EEG hypoactivity. This implies that ketogenic diets produced more acute periods of hypoactivity than did controls. It is also possible that these dips represent short-lived spreading depression events. To that end, current source density analysis was performed to determine whether spontaneous spreading depression events were the cause. Current source density analysis showed no cortical spreading depression events in either control or ketogenic contralateral recordings. Since no spreading depression events were measured, and since the primary data revealed the lack of any discrete explanatory event, there is no explanation for this finding.

**Future research:**

This research found no obvious differences in basal excitability, a finding consistent with data that showed that animals exposed to a ketogenic diet did not differ from controls based on excitability measures or paired-pulse measures, but did exhibit a decrease in synaptic potentiation in the hippocampus (Blaise et al., 2015). The next step in this research would be to evaluate whether such a discrepancy is the case in the cerebral cortex by evaluating paired pulse and synaptic potentiation measures. Moreover,
it would be very useful to evaluate whether the induction of stroke was delayed or potentiated with a ketogenic diet by recording ipsilaterally to the stroke.

Immunohistochemical studies can be performed on the brains gathered from this experiment to investigate differences in neuronal populations between groups. It is unclear whether glutaminergic and GABAergic respond similarly to changes in metabolism, and specific stains could provide valuable information to that question.

Because changes in contralateral hemisphere activity following stroke have not be well characterized, it is worthwhile to continue analysis of the primary EEG data. Focus should remain on changes in frequency and amplitude of contralateral EEG activity during stroke. The cellular changes concurrent with the observed dips should also be characterized. It is possible that these dips correspond to metabolic insufficiency without associated CoSD, and should be evaluated further.

Additionally, some studies have evaluated the influence of KD on hippocampal frequencies above 150Hz (Simeone, Samson, Matthews, & Simeone, 2014). It is possible that our design missed valuable information about neuronal frequency changes in the 150-200Hz range. Future studies could extend the frequency ranges, and the location of brain recording.
REFERENCES


CURRICULUM VITAE

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EDUCATION

Boston University School of Medicine – Boston, MA
Master of Science in Medical Sciences, 2016 (anticipated) GPA: 3.96

University of California Los Angeles – Los Angeles, CA
Bachelor of Science in Physiological Sciences, December 2012

EXPERIENCE

Dr. Jarrett Rushmore Neuroscience Lab-Research Assistant
August 2015-Current

• I work part-time as a research assistant in Dr. Jarrett Rushmore’s neuroscience lab at Boston University Medical School. This research focuses on cortical spreading depression in a rat model, and investigates the potential for therapeutic interventions using transcranial magnetic stimulation, and diet manipulation. I am writing my thesis on the implementation of ketogenic diets in relation to stroke events, which is a project that I proposed given my interest in metabolism and cognition.

Boston Medical Center, Otolaryngology-Medical Scribe
August 2015-Current

• I work part-time as a medical scribe in the Department of Otolaryngology at Boston Medical Center. I utilize the program “EPIC” to input patient data, and input the physicians’ dictations. This position has been given me incredible clinical exposure, and has improved my understanding of medical terminology and practices.

ESM Group- Academic Tutor/Mentor
January 2013-June 2014

• I tutored high school students for the SAT/ACT exams, SAT subject tests, and AP exams. I also tutored for weekly coursework in Biology, Pre-Calculus, Chemistry, English and History, and helped with college counseling.
UCLA Cardiovascular Lab - Dr. Yibin Wang  
*Research Assistant, June 2012-January 2014*

- Dr. Yibin Wang’s lab studies various cardiovascular responses to stress stimuli. I collected mice tails in a barrier facility, isolated and purified DNA from those tails, ran polymerase chain reactions and used gel electrophoresis to identify specific genes of interest. Additionally, I managed Dr. Wang’s calendar, maintained correspondence with visiting researchers, planned events, and maintained records of all mouse data.

UCLA Psychophysiology Lab - Dr. Cindy Bradbury  
*Research Assistant, June 2011-June 2012*

- I led patients with schizophrenia in Cindy Bradbury’s lab through a protocol that measured parameters like cortisol secretion, sweat gland activity and EEG activity in response to stressful situations. This included hooking up EEG and EKG equipment, and verbally instructing subjects throughout the study. Inherent in the position was a need to empathize and be patient with uncomfortable or uncooperative subjects, and be comfortable with consistent personal interaction.

VOLUNTEER/ORGANIZATION INVOLVEMENT

UCLA-KASEO (Now LASEO)  
*Public Relations Officer, Academic Tutor, August 2010-July 2012*

- KASEO (Koreatown All-Star Educational Outreach) is a program where UCLA students volunteer to tutor and mentor disadvantaged youth from diverse ethnic and socio-economic backgrounds in Los Angeles. I joined KASEO during my junior year at UCLA because I wanted to participate in a meaningful community service activity. In my senior year, I became more involved in KASEO as a Public Relations officer, with a goal of increasing awareness of KASEO on the UCLA campus, boosting student participation and increasing diversity within the program. I helped to coordinate fundraisers, recruitment and marketing events, as well as continued to volunteer as a tutor and mentor. KASEO is now “LASEO,” (Los Angeles Students Educational Outreach) which is a reflection of the increased diversity I aimed to attain.

UCLA-LAMBDA CHI ALPHA  
*Founding member, Academic Chair, Member of “High Zeta” (House Government)-March 2009-December 2012*

- During my freshman year at UCLA I helped recolonize the Lambda Chi Alpha fraternity along with 27 others, and a few hopeful alumni. There are now over 100
members, all of whom embody the values we originally set forth to promulgate. I was Academic Chair my sophomore and junior year and participated on our governing body "High Zeta" to help monitor internal and external affairs. We recently won the "Grand High Alpha" award at our national convention, designating us as the best chapter across the nation in regards to community service, academic record, and involvement with nationals.

SKILLS

- **Lab**- Mouse care, pipetting, DNA purification, PCR, electrophoresis
- **Computers**- Matlab, Zotero, Adobe InDesign, SPSS, Excel
- **Hobbies**- Music, squash, basketball, reading