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EFFECTS OF THE KETOGENIC DIET ON SPATIAL MEMORY AND LONG-TERM POTENTIATION IN THE CA1 REGION OF THE HIPPOCAMPUS IN YOUNG RODENTS

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Honors Thesis

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by
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ABSTRACT

EFFECTS OF THE KETOGENIC DIET ON SPATIAL MEMORY AND LONG-TERM POTENTIATION IN THE CA1 REGION OF THE HIPPOCAMPUS IN YOUNG RODENTS

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Department of Cell Biology and Physiology

Bachelors of Science

The ketogenic diet (KD) originated as a treatment for epilepsy nearly a century ago, though its neurological effects are not completely understood. In recent years the diet has resurged as a weight loss aid, reinvigorating research investigating its effects on the nervous system [2]. Notably, the KD alters the concentration of glucose and ketones in the brain, and recent research suggests that the elevated ketone concentration, in the form of beta-hydroxybutyrate, induced by the diet enhances recognition memory and mitochondrial efficiency in the hippocampus [1]. To further explore the effects of the KD, we examined its cognitive effects on spatial memory and synaptic plasticity in the CA1 region of the hippocampus.

We utilized rodents as models of learning and memory, and investigated our question through the use of behavioral and electrophysiological assays. The rodents were divided into one of two treatment groups, either a 3-4 week high lipid diet, enriched with a ketone ester, to increase ketone bodies *in vivo*, or bathing hippocampal slices in a controlled amount of beta-hydroxybutyrate (BHB)-enriched artificial cerebrospinal fluid

(ACSF) to produce a higher concentration of ketones than was produced in rodents *in vivo*. Following the diet, its effects on spatial memory were measured by performance in the Morris Water Maze, and effects on synaptic plasticity in both the diet and enriched-ACSF groups were determined by theta-burst induced hippocampal CA1 Long-Term Potentiation (LTP) field electrophysiology experiments. We observed no difference in young mice, but an increase of LTP in young female rats.

ACKNOWLEDGEMENTS

This thesis was greatly supported by Dr. Jeff Edwards, and other members of the Edwards Lab. Additionally, the work of Erin Saito and Dr. Ben Bikman provided a critical foundation in methods and resources for this research. The resources provided by the Department of Cell Biology and Physiology, the Neuroscience Center, the College of Life Sciences, and the Honors program were also instrumental in the execution of this project.

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Introduction

The ketogenic diet (KD) has been used as a treatment for epilepsy for over a century, and though much research has been conducted regarding the effects of the diet on the brain, little has been done investigating its effects on synaptic plasticity [3]. Synaptic plasticity refers to the ability of synaptic connections in the brain to be strengthened or weakened in response to experience. The ebb and flow of this potentiation and depression defines our current understanding of learning and memory in the hippocampus [4]. Of particular interest in this field is Long-Term Potentiation (LTP), which is associated with the strengthening of synaptic connections and simultaneous strengthening of circuits and memory [5]. Additionally, the degree to which these synapses are strengthened is associated with positive effects in learning and memory [6]. The decline in these connections is frequently observed in pathologies of the brain, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) [7, 8].

Diseases of neurodegeneration, such as AD and PD, have become increasingly pervasive in the last few decades [9, 10]. Symptoms of these diseases include dementia, and decreased faculties of learning and memory [11]. As these diseases become increasingly common, treatments that can relieve their symptoms grow in demand [9, 10]. The KD has been investigated as a potential treatment, but its efficacy has yet to be proven [12, 13]. Additionally, the precise effects of the diet on synaptic plasticity are a matter of contention [14, 15].

The primary effect of the ketogenic diet is its alteration of the concentrations of the brain's two primary sources of energy: glucose and ketones [16, 17]. We are particularly interested in the elevations of beta-hydroxybutyrate (BHB) levels, as it has

been associated with neuroprotective effects [18]. Our study utilizes a recently established ketone ester supplemented ketogenic diet, that has been shown to have efficacy in establishing and maintaining an effectively high BHB concentration in rodents [1]. Additionally, to further explore the effects of high ketone concentration we utilized an acute in vitro treatment of BHB-enriched artificial cerebrospinal (ACSF), to further elevate ketone concentrations in the brain beyond what we observed in rodents on the diet. In each of these cases, we performed field electrophysiology experiments involving theta-burst induced hippocampal CA1 LTP to determine their effect on synaptic plasticity in this region.

The hippocampus is a region of the brain that is highly associated with learning and memory, which are greatly impacted by diseases of neurodegeneration [19, 20]. Within the hippocampus, the CA1 region has been associated with spatial and recognition memory, both of which are affected by the symptoms of AD and PD [11, 21, 22]. The effects of the ketogenic diet on recognition memory have already been explored, but its effects on spatial memory are not well established [1, 23, 24]. As a result, we selected the CA1 region of the hippocampus as our area of focus in our study of synaptic plasticity, and we also implemented behavioral assays to measure spatial memory. We selected the Morris Water Maze assay as it requires minimal training and is an effective and minimally stressful test of spatial memory that doesn't require fasting prior to the assay [25]. This is especially important as fasting can alter the concentrations of glucose and BHB in way that we cannot control for [34].

The combination of behavioral and electrophysiological experiments provides us with a powerful practical and mechanistic view into the hippocampal effects of the KD.

Additionally, AD and PD are biased with regards to sex and age [9, 10, 26, 27]. There is also evidence that the ketogenic may have differential effects depending on these two variables as well [28, 29]. As a result, we implemented a protocol of methods and analysis to especially determine the effects of age and sex on our results. Finally, to ensure scientific rigor of experiments researchers were blinded as to which treatment group they were analyzing. We also used sample sizes of at least 10 for each treatment group and in controls.

Our results thus far demonstrate that the ketogenic diet has no effect on the magnitude of LTP or performance in the Morris Water Maze in young mice. Similarly, the use of BHB-ACSF had no effect on LTP in young mice. These results were consistent regardless of sex. However, application of BHB-ACSF in vitro significantly enhanced LTP in young female rats. These results represent the first stage of our experimentation. We are currently in the process of examining the effects of both treatment types on spatial memory and LTP in adult mice. However, the data collected thus far suggests that the ketogenic diet may have effects on memory and synaptic plasticity that are dependent on age and sex. As a result, the utility of the ketogenic diet as a potential treatment for the symptoms of neurodegeneration warrants further investigation. Additionally, due to recent work demonstrating improved mitochondrial efficiency in adult mice on the ketogenic diet, we predict that LTP and spatial memory will be enhanced in adult mice on the ketogenic diet. We predict that the ketogenic diet and/or BHB treatment will have little effect on synaptic plasticity and spatial memory in young animals, but that it will significantly improve both spatial memory and long-term potentiation in adult animals both on the diet and those whose slices are treated with BHB-enriched ACSF.

Methods

Our research was conducted in accordance with National Institutes of Health Guide for the Care and use of Laboratory animals and was approved by Institutional Animal Care and Use Committee (IACUC) of Brigham Young University. Additionally, many of our methods are similar to those published previously [1, 30].

Flow Chart of Methods -

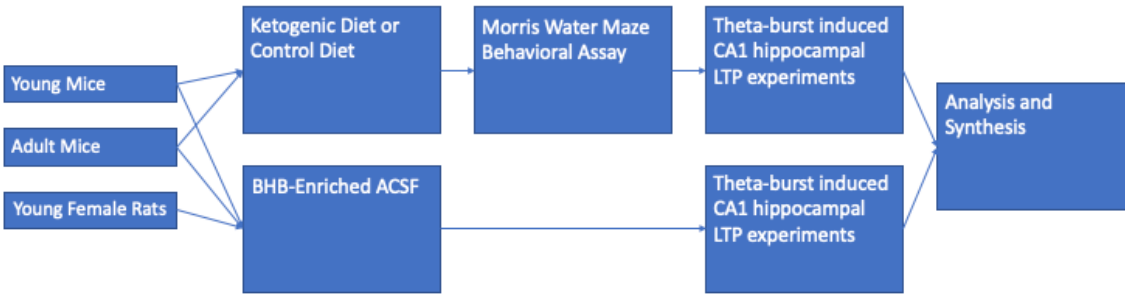

Table 1 – Above is depicted a flow chart of the essential methods for each treatment group.

Experiments with Young Mice on the Ketogenic Diet - We obtained young (4-6 week old) CD1 mice from Jackson Laboratories. These animals were kept at 22 +/- 1 degrees Celsius, 60-70% humidity, group-housed, and kept on a 12-hour light-dark cycle. We divided the mice randomly into two groups, and each was supplied with ad libitum access to food and water. One group was supplied with a control rodent diet purchased from LabDiet (5001). While the other was given a ketogenic diet (KD) that was supplemented with an exogenous ketone ester. The KD was composed of the following: lard-based keto paste (Bioserv (F3666)), no sugar-added JIF peanut butter to improve palatability, and R,S-1,3-butanediol acetoacetate diester (ketone ester, KE; Disruptive Enterprises). By

weight, the diet was composed of 90% keto paste, 5% peanut butter, and 5% ketone ester. This composition corresponds to a macronutrient composition of 90% kcal fat, 4.7% kcal protein, 2% kcal carbohydrate, and 3.3% kcal ketone ester. The diet was prepared twice a week, and was given in sufficient amounts, according to weight to provide ad libitum access to food.

	Control Diet	Ketogenic Diet
Component	kcal%	kcal%
Fat	13.4	90
Protein	28.7	4.7
Carbohydrate	57.9	2
Ketone Ester	0	3.3

Table 2 – Macronutrient composition of control and ketogenic diets by kcal%

In the first week of the diet period, animals were weighed every day to prevent excessive weight loss. Animals that lost more than 20% of their initial body weight were excluded from the study. Following the first week, animal's weight was measured every week. Additionally, before the diet period and every week thereafter we measured blood glucose and blood ketone levels in all mice using tail tip amputation and tail massage, (measured by Precision Xtra blood glucose and ketone meters (Abbott)), to ensure that mice on the diet underwent ketosis. Additionally, prior to behavioral and electrophysiological experiments we ensured that female rodents were not in proestrus, as LTP is enhanced in female rodents during that time [31].

Following the diet period, we conducted Morris water maze behavioral assays to measure the effects of the diet on spatial memory. It is important to note, that one distinct

advantage of the Morris water maze over other assays of spatial memory such as the radial arm maze, is that it does not require food as motivation. Such assays typically implement a period of fasting that can alter blood ketone levels and thus could confound our results [32]. The Morris water maze experiments were conducted in a round, plastic, container filled with non-toxic drinking water (22-25 degrees Celsius). Water was then clouded with non-toxic tempura paint to opacity. Mice were allowed to acclimate to the testing environment for 20 minutes prior to the trial, and observers stood in the same locations for all training days and trials. Additionally, a curtain surrounded the pool so the mice could not use cues from outside the testing environment. The mice underwent a two-day training period where the platform was placed in the center of pool, and mice were placed in the pool so they could learn the pattern of finding the platform and rescue. Mice were allowed to swim freely for 60 seconds at which point they were guided to the platform. If mice did not stay on the platform for 10 seconds they were removed from the pool at 120 seconds. Mice were then returned to their cages to dry via heating pad.

Following the training period, behavioral data was acquired for a period of 4 days. The pool was set up the same as in training except that the spatial cues (shown in figure 1) were attached, and the platform remained hidden .5-1 cm below the surface of the water. Mice were again acclimated to the testing environment. Mice were recorded by GoPro as they performed the test. Mice were allowed 60 seconds to attempt to find the platform after which time they were guided there. They were removed from the pool if they did not remain on the platform for a minimum of 10 seconds during the 120 second trial period. Mice were placed in the pool at semi-randomly determined drop locations. The trial was then repeated for each drop location with 20 minutes between trials. This

protocol was repeated for 4 days, before final acquisition on day 7. The protocols explained above were again implemented except that the mice were allowed 45 seconds to find the platform, and time spent in the quadrant of the platform was measured. From these assays, we measured time in correct zone, time to find platform, distance travelled, and velocity of travel of the mice. This data was then analyzed for each day of acquisition.

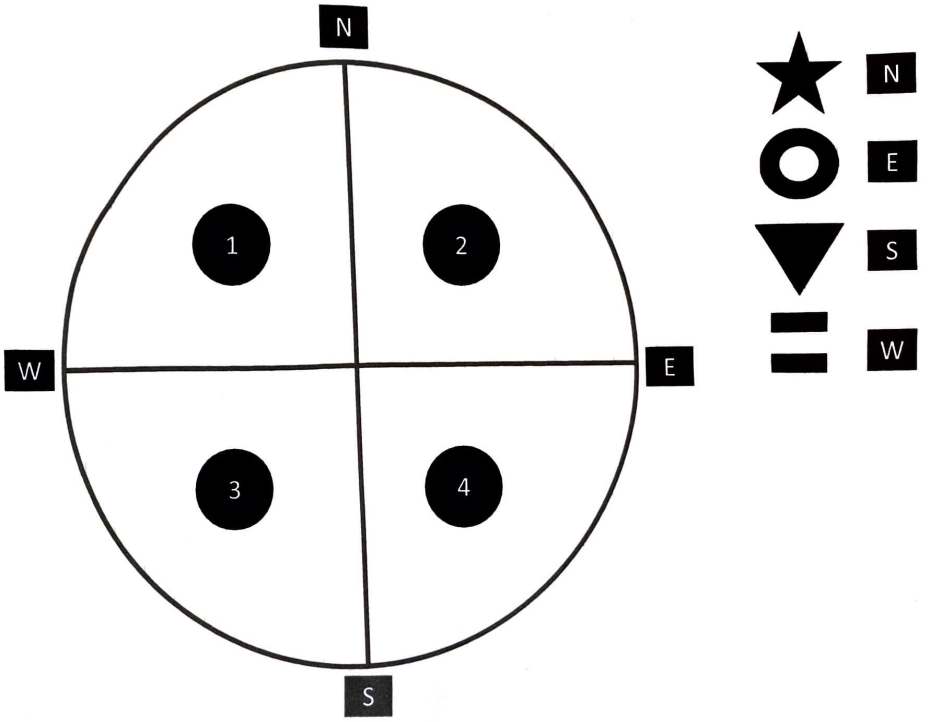

Figure 1 - Above is a depiction of the Morris water maze, note than in this depiction, only 1 platform is available at a time, but the location may be changed.

Trial	Training Day 1-2	Acquisition Day 1 Drop Locations	Acquisition Day 2 Drop Locations	Acquisition Day 3 Drop Locations	Acquisition Day 4 Drop Locations
Trial 1	Semi-Random	N	W	S	E
Trial 2	Semi-Random	S	E	N	W
Trial 3	Semi-Random	E	S	W	N
Trial 4	Semi-Random	W	N	E	S

Table 2 – Drop locations for each trial and day of acquisition

Following the behavioral assays, we prepared brain slices in accordance with methods published in [31]. Mice were anesthetized in a vapomatic chamber with isoflurane and were then decapitated via guillotine. Subsequently, brains were rapidly extracted and maintained in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) of the following composition: 119 mM NaCl, 26 mM NaHCO3, 2.5 mM KCl, 1 mM NaH2PO4, 2.5 mM CaCl2, 1.3 mM MgSO4, and 10 mM glucose saturated with 95% O2 and 5% CO2. The pH of the ACSF was 7.4. We obtained the salts in this solution from Sigma-Aldrich (St. Louis, MO, USA), Mallinckrodt-Baker (Phillipsburg, NJ, USA) or Fisher Scientific (Waltham, MA, USA) and dissolved them in double-distilled water (ddH2O). Using a vibratome (Leica), we cut coronal hippocampal slices (400 uM) in ice-old ACSF before dissecting and transporting slices to room-temperature oxygenated ACSF where they were maintained for a minimum of two hours before experiments began. They were then transferred to a submerged recording chamber and continually perfused with oxygenated ACSF (average 30 degrees Celsius, and flow rate of 2-3 mL/min).

Following this period, we performed field electrophysiology experiments via two-electrode stimulation/recording in the CA1 region of the hippocampus. A bipolar stainless steel stimulating electrode was placed in the stratum radiatum of the CA1 region of the hippocampus to stimulate the Schaffer Collateral pathway with a current of 10-20 uA every 10 seconds for a period of 15 minutes while we recorded corresponding voltage responses. Electrode position and stimulation intensity were adjusted to evoke an excitatory post-synaptic potential (EPSP). Recordings were obtained utilizing current clamp mode of an Axopatch 200B or MultiClamp 700B amplifier (Molecular Devices). These were measured using borosilicate glass patch pipettes (2-3 Megaohm) filled with 1 M NaCl. Following 15 minutes of baseline, we applied theta burst stimulation (two bursts with each burst consisting of 10 sets of 5 pulses, each pulse lasting 100 usec and applied at 100 Hz with 200 ms between each set, and a 20 second delay between each burst) to induce LTP in the slices. Following stimulation, we recorded EPSPs for 60-90 minutes to characterize the magnitude and appearance of the potentiated response in hippocampal slices from mice both on the KD and on the control diet.

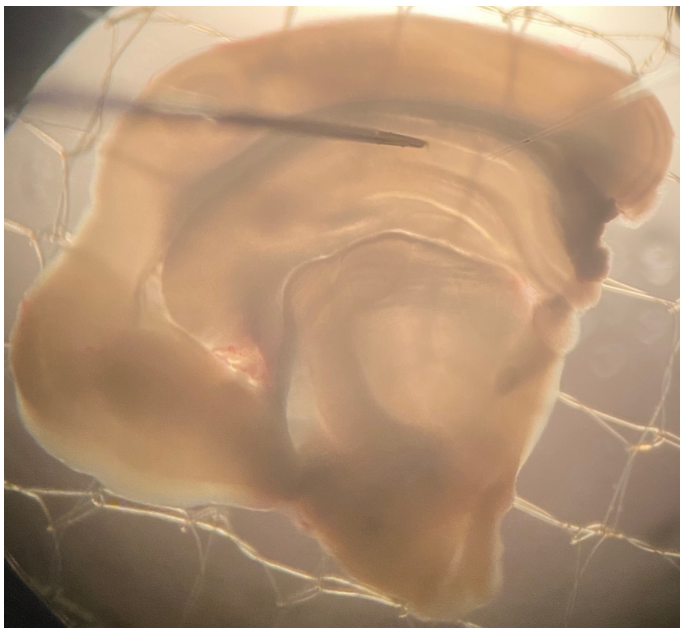

Figure 2 – Coronal hippocampal brain slice with recording (right) and stimulating (left) electrodes inserted in the CA1 region as described in the methods.

After data collection, our results were analyzed using ClampFit 10.7 software (Molecular Devices). We obtained EPSPs slope values and those measured every 10 seconds were averaged to 1 minute intervals. Slope values were then normalized and baseline and post-stimulus values for each group were compared. We tested for significance at 26-30 and 41-45 minutes post theta-burst stimulus using a two-tailed type 3 t-test. Significance is defined as having a p-value less than 0.05. Microsoft Excel and Origin (North Hampton, MA) were used to analyze, organize, and graph our findings. Additionally, traces were obtained and analyzed to observe characterization of responses in each group.

Experiments with Young Rodents using BHB-Enriched ACSF – We obtained female Sprague-Dawley rats and CD1 mice from Jackson Laboratories**.** Animals were then housed according the protocols outlined in the *Experiments with Young Mice on the Ketogenic Diet* with the exception that all rodents were kept on a control diet [1].

Hippocampal slices were then obtained from the rodents according to the protocol outlined in the previous section. Except that we randomly selected half of the animals' hippocampal slices to be cut and maintained in our novel BHB-Enriched ACSF which allowed us to precisely control BHB concentration without variation at a higher concentration than was observed in the mice on the diet. Normal ACSF has a macronutrient concentration of 11mM glucose and 0mM BHB, but we prepared our BHB-enriched ACSF with 7.5 mM BHB and 2.5 mM Glucose. The precise concentration of the BHB-enriched ACSF is as follows: 119 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 2.5 mM glucose, and 7.5 mM BHB saturated with 95% O_2 and 5% CO_2. The pH of the ACSF was 7.4. We obtained the salts and BHB in this solution from Sigma-Aldrich (St. Louis, MO, USA), Mallinckrodt-Baker (Phillipsburg, NJ, USA) or Fisher Scientific (Waltham, MA, USA) and dissolved them in double-distilled water (ddH_2O). Following extraction, slices were bathed in each respective solution for a minimum of two hours prior to experiments, after which we conducted field electrophysiology experiments using theta-burst induced CA1 LTP as explained above, and data were analyzed as previously described.

Results

Experiments with Young Mice on the Ketogenic Diet –

Figure 3

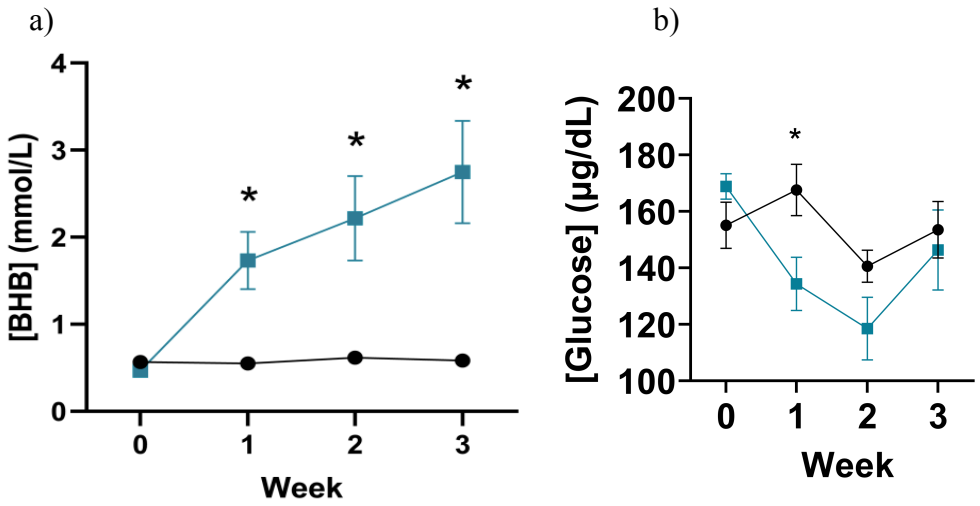

Figure 3.1 – *Blood ketone concentration increased in young mice on the ketogenic diet*. **a)** During the three-week period the young mice were on the ketogenic diet, their blood BHB concentration increased. BHB concentration reached approximately 3mmol/L after 3 weeks, compared to .5 mmol/L in control (control n=12, KD n=11). **b)** Blood glucose concentrations remained the same during the 3-week diet period for both control and KD mice (control n=12, KD n=11).

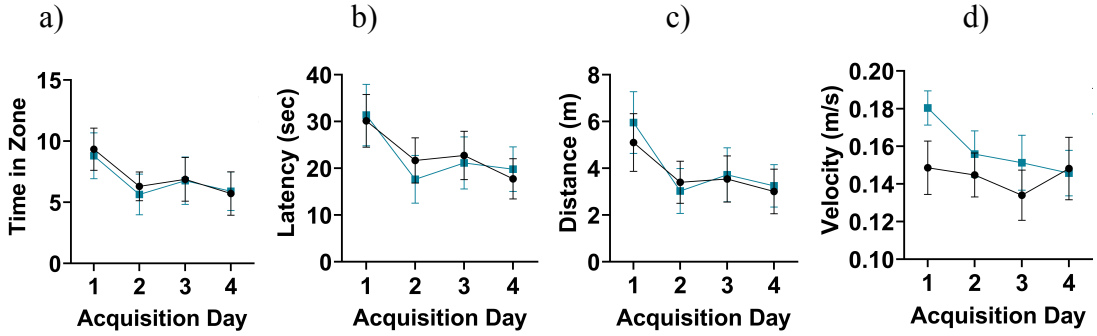

Figure 3.2 – *The 3-week ketogenic diet period did not affect spatial memory in young mice.* **a)** Time in the same quadrant as the platform decreased across acquisition days (as expected) but not between groups on the control vs. ketogenic diet (control n=12, KD n=11). **b)** Time to find the platform also improved across acquisition days but not between groups (control n=12, KD n=11). **c)** Distance swam decreased across acqusition days but not between groups (control n=12, KD n=11). **d)** Velocity of swimming did not change acqusition days nor between groups (control n=12, KD n=11).

a)

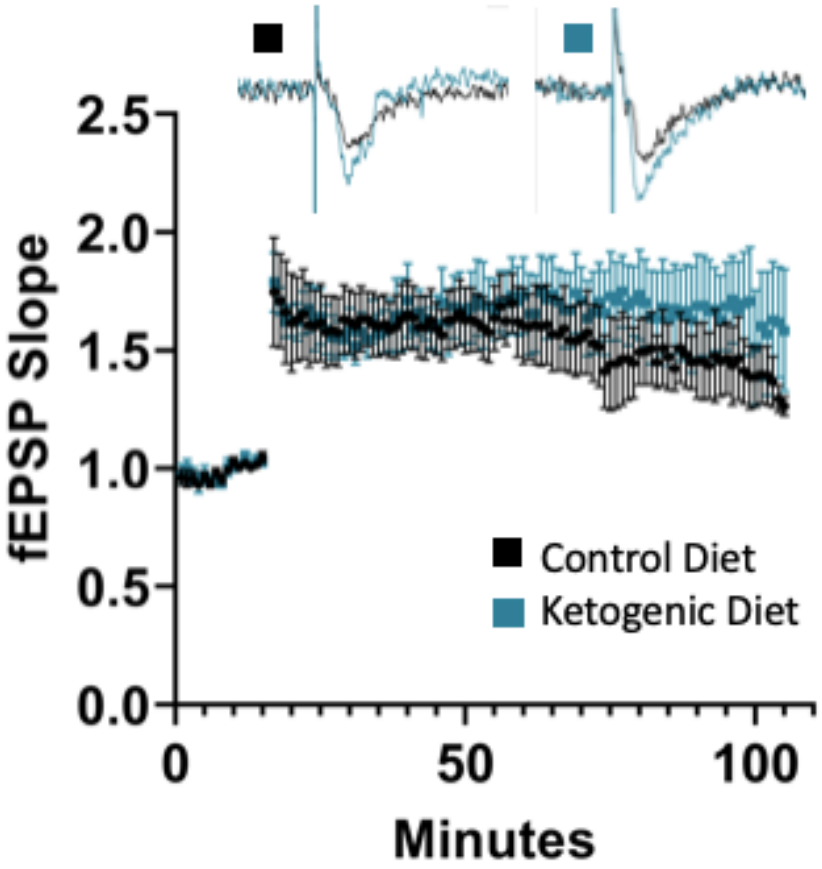

Figure 3.3 – *The 3-week ketogenic diet did not affect Long-Term Potentiation in young mice.* **a)** Slices obtained from mice on a 3-week ketogenic diet showed no difference in LTP compared to slices obtained from mice on a normal diet (173% ± 14% vs 160% ± 13%) from both 26-30 and 41-45 minutes post-theta ($p = 0.5556$; $p = 0.9702$). (control n=5, KD n=5)

Following the 3-week administration of the ketone-ester supplemented ketogenic diet we conducted Morris water maze behavioral assays and field electrophysiology experiments using theta-burst induced Hippocampal CA1 LTP to determine the effects of the diet on spatial memory and synaptic plasticity in young mice.

Over the course of the diet period the mice on the ketogenic diet experienced an increased blood concentration of BHB compared to the mice on the control diet. This is evidence that the mice indeed underwent ketosis. Additionally, the mice on the ketogenic diet experienced no difference in blood glucose concentration over the course of the diet period (Figure 3.1).

Following the diet period, we conducted behavioral Morris water maze assays to assess spatial memory in accordance with the protocols outlined in the methods section. We expect that the mice will learn and remember the spatial cues over the course of the four days of acquisition, and we can thus correlate that improvement to spatial memory. Both groups of mice improved their performance in the assay as reflected by the decreased time in zone, decreased latency, decreased distance swam, and similar swimming velocity. However, there was not a significant difference in performance in any of these categories between the mice on the ketogenic diet and mice on the control diet. This suggests that the ketogenic diet did not improve spatial memory in young mice (Figure 3.2).

After the behavioral assays, coronal hippocampal slices were extracted as outlined in the methods, and we performed theta-burst induced CA1 LTP field electrophysiology experiments to assess the effect of the ketogenic diet on synaptic plasticity, or more specifically long-term potentiation. In both the control and treatment groups we observed potentiation of the EPSPs, but there was no difference in magnitude or characterization between the two groups (Figure 3.3).

Experiments with Young Rodents using BHB-Enriched ACSF –

Figure 4

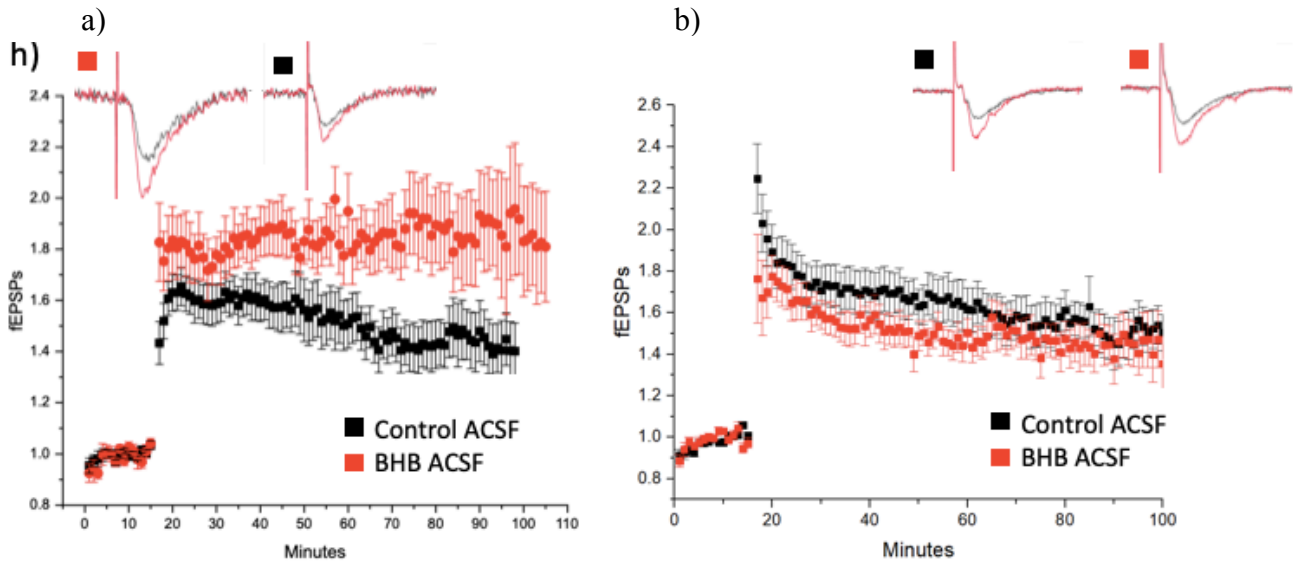

Figure 4.1 – *BHB-Enriched ACSF increased Long-Term Potentiation in young female rats, but not in young mice.* **a)** Slices obtained from young female rats bathed in BHB-enriched ACSF showed a significant increase in LTP compared to slices bathed in regular ACSF (190% ± 13% vs 150% ± 9%) from both 24-28 and 55-59 minutes post-theta (p = 0.0326; p = 0.0138 (control n=13, BHB n=13) **b)** Slices obtained from young mice bathed in BHB-ACSF showed no significant difference in LTP compared to slices bathed in regular ACSF (150% ± 8% vs 166% ± 11%) from both 26-30 and 41-45 minutes post-theta (p = 0.2106; p = 0.2367). (control n=12, BHB n=10).

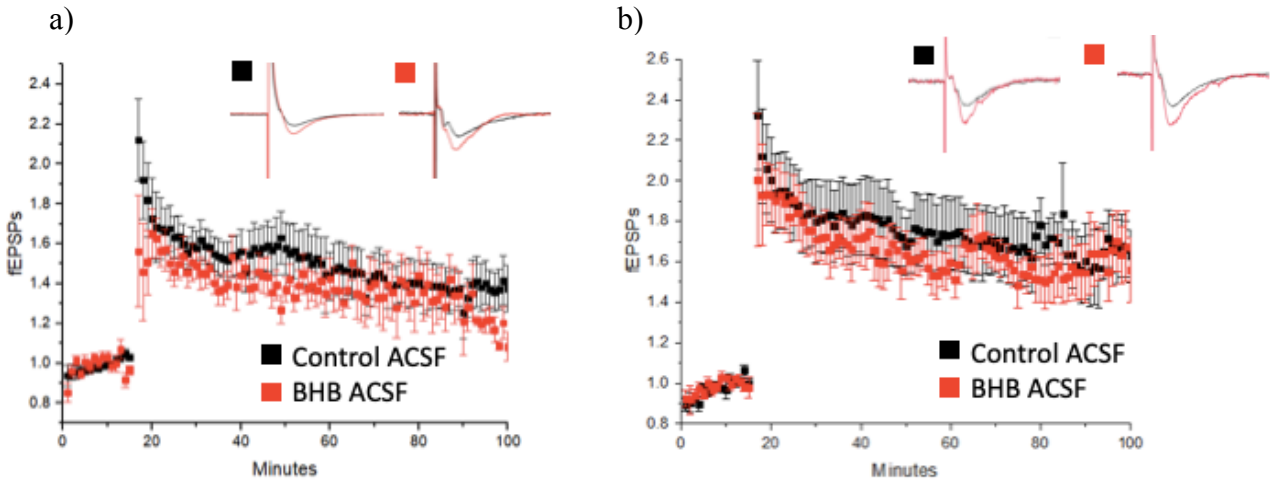

Figure 4.2 – *BHB-Enriched ACSF had no sex-specific effects on Long-Term Potentiation in young mice.* **a)** *Slices bathed in BHB-ACSF from young female mice showed no significant difference in LTP compared to slices bathed in regular ACSF* (141% ± 12% vs 154% ± 13%) from both 26-30 and 41-45 minutes post-theta (p = 3472; p = 0.6806) (control n=4, BHB n=5). **b)** *Slices bathed in BHB-ACSF from young male mice showed*

no significant difference in LTP compared to slices bathed in regular ACSF (160% ± 11% vs 177% ± 18%) from both 26-30 and 41-45 minutes post-theta ($p = 0.4870$; $p= 0.3895$). (control n+7, BHB n=5).

We also conducted field electrophysiology experiments on coronal hippocampal slices obtained from rodents on the control diet. However, to examine the effects of BHB concentration that is higher than can be attained in vivo we employed a BHB-enriched ACSF as explained previously. The mice reached about 3 mmol/L BHB in vivo, compared to 7.5 mmol/L in the BHB-ACSF.

Using theta-burst induced hippocampal CA1 long-term potentiation field electrophysiology experiments we collected data for both hippocampal slices perfused with normal ACSF and BHB-ACSF. In young female rats, both groups experienced potentiation in response to stimulus, but the slices bathed in the BHB-ACSF experienced a huge increase in the magnitude of LTP. Additionally, in terms of characterization, the EPSPs from the slices bathed in control ACSF potentiated, levelled out, and then slowly began to decrease, whereas the EPSPs from the slices bathed in the BHB-ACSF potentiated, and then continued to increase in magnitude for the entire duration of data collection. However, we did not see the same result in slices from young mice. Both those bathed in control and BHB-ACSF potentiated, but there was no difference in magnitude or characterization between slices bathed in each ACSF (Figure 4.1).

After we obtained the results explained above, we analyzed the data from the young mice to see if there were sex-dependent differences (As notably the data from the young rats included only young female rats). There were no significant differences in magnitude or characterization of post-stimulus EPSPs for either sex when comparing mice on the ketogenic versus the control diet (Figure 4.2).

Discussion

Summary – The ketogenic diet did not affect spatial memory or LTP in young mice. Application of a BHB-enriched ACSF enhanced LTP in young female rats, but not in young mice. We hypothesized that the ketogenic diet would not enhance LTP or improve spatial memory in young mice, as there is research that suggests that the brain's bioenergetics are more greatly impacted by metabolism and sources of macronutrients in adults compared to young animals [38].

Discussion – The most surprising element of our results was the massive increase in LTP we observed in young female rats. We actually began our study in this line of research as a result of that surprising finding. This could just be a result of species differences, but here is an exciting precedent for this observation [36]. One study demonstrated that there was no difference in the magnitude and characterization of LTP in the CA1 region of the hippocampus in young female rats compared to adult female rats [35]. Typically, the magnitude of LTP is diminished in adult animals when compared to young animals [37]. The prevailing theory for why young and adult female rats have similar LTP is that the prevalence of NMDA receptor subunits NR1 and NR2A are equivalent in female rats regardless of age, but young male rats have more subunits than adult male rats [35].

Young female rats experienced enhanced LTP in the presence of elevated BHB concentrations, while young mice did not. This finding suggests that young female rats may experience this increase because their physiology in the CA1 region of the hippocampus is more similar to adults. Additionally, the prevalence of NR1 and NR2A NMDA receptor subunits, which are vital for LTP, may be instrumental in the

mechanism of enhanced memory observed in adult rodents on the ketogenic diet [35, 38] This finding is exciting, but is yet to be fully affirmed, as we have not conducted our experiments with adults.

In mice, we observed that LTP and spatial memory were not enhanced in young mice by an elevated BHB concentration. As mentioned earlier, the ketogenic diet has been around for a long time, but it's increase in popularity in recent years has sparked a resurgence into the potential benefits and/or negative impacts of the diet. One of the most interesting findings to come out of that research was that elevated BHB concentrations may be correlated to neuroprotective effects [12]. However, the exact nature of those neuroprotective effects remains unknown [2]. It is vital to note that the neuroprotective effects of the ketogenic diet are not fully established, and its effects on cognition remain disputed [18, 24, 40]. It is also interesting in the context of our studies, that the majority of studies on this topic have involved juveniles and adolescents, and not adults [2]. One of the primary criticisms of studies showing beneficial effects of the ketogenic diet on cognition is the lack of a well-defined mechanism [2]. To provide further context for our findings, I will summarize some of the insights that we have on the effect of the ketogenic diet on cognition and the hippocampus.

A recent study at BYU demonstrated that the ketogenic diet enhanced mitochondrial efficiency and complex V expression in the CA1 region of the hippocampus [1]. This suggests that altered mitochondrial function in the hippocampus is one of the mechanisms of the ketogenic diet. Similarly, the ketogenic diet also inhibits the formation of free radicals in the respiratory chain complex [41]. This antioxidant capability increases activity of glutathione peroxidase, which is correlated with

neuroprotective effects [12, 42]. Similarly, the ketogenic diet may reduce reactive oxygen species in the brain, which in turn reduces inflammation and beta-amyloid concentrations [33]. These metabolic effects relate to the general theory that the ketogenic diet's neuroprotective effects are due to the enhanced ATP production allowed by the diet [3]. Apart from metabolism there are a few other important mechanisms that may be involved.

First, the ketogenic diet has long been shown to have anticonvulsant properties essential to its function as an epilepsy treatment [3]. Additionally, in the case of Alzheimer's disease, glucose metabolism is impaired, so providing an alternative source of energy in high concentrations may be relevant to that particular case of neuroprotection [13]. The ability of the ketogenic diet to provide an alternative source of energy when glucose use and uptake is impaired is also relevant to the pathology of dementia in general [43].

These findings offer mechanistic insight, but present some notables holes in our understanding. As a result, synthesis between previous research, our research and future research is essential for seeing the whole picture of the impact of the ketogenic diet on cognition. Behavioral assays are an effective measure of the in vivo effects of a treatment on cognition, and electrophysiology takes us one step deeper, allowing us to observe a physical effect that has been strongly associated with the behavior. That being said, we do not yet know how any of the changes in synaptic plasticity are brought about. We hope to lay the foundation for future discovery by connecting synaptic plasticity to the neuroprotective effects of the ketogenic diet. Incorporating synaptic plasticity into our current view of the effects of the ketogenic diet provides a link between the more

mechanistic discoveries explained previously relating to metabolism. Our current interpretation of the data is limited in that we have not completed the trials with adults, but we hypothesize that the enhanced mitochondrial efficiency (and its effects on ATP availability, and reduced free radicals) observed in adults on the diet enhances synaptic plasticity, which then leads to improvements in learning and memory. Additionally, we propose that these improvements in cognition are not observed in young animals, as dependence on alternative energy sources to compensate for decreased glucose use is unlikely in young animals [44].

The goal of this research is to further establish the bridge between biology and behavior with regards to the neuroprotective effects of the ketogenic diet. One of the effects of neurodegeneration is impaired synaptic plasticity, and enhancing synaptic plasticity is also correlated to neuroprotective effects [7]. As a result, our overall purpose is to determine whether enhanced synaptic plasticity evoked in adult animals by an elevated BHB concentration is a potential factor in the ketogenic diet's neuroprotective effects. Additionally, we hope to establish a connection between behavior and our findings, and as a result we are investigating the consequences of this altered synaptic plasticity through assays of spatial memory. That being said, we have not yet completed our research involving the adults, and thus we cannot yet draw conclusions or make comparisons with that group.

Conclusions

Our 3-week ketone ester supplemented ketogenic diet does not affect spatial memory or long-term potentiation in young mice. Application of our novel BHB-enriched ACSF enhances LTP in young female rats, but not in young mice.

Future Directions

Prior to publication and a complete analysis of this project we will complete our experimental trials involving adults. The adult mice are currently on the ketogenic diet, and will be prepared for testing in mid-March. At that point, we will analyze spatial memory through the Morris water maze behavioral assay, and synaptic plasticity through theta-burst induced CA1 LTP experiments using field electrophysiology. Then we will test the effects of the BHB-enriched ACSF on LTP using that same protocol. Once these experiments are completed we will be able to analyze age and sex effects of the ketogenic diet and elevated BHB concentrations on LTP and spatial memory. We will then be able to draw conclusions relating to the involvement of synaptic plasticity in the neuroprotective effects of the ketogenic diet, and we will broaden our understanding of the ketogenic diet as a potential treatment for diseases of neurodegeneration.

Additionally, we do not have plans to look into the following, but I would be interested in investigating another couple of ideas. First, to deepen our understanding of the role of synaptic plasticity in the neuroprotective effects of the ketogenic diet I would be interested in looking at the effects of elevated BHB concentration on long-term depression and potentially depotentiation. I would also be interested in going back and

looking at our data from young animals, to see if smaller differences in animal age affected the impact of the ketogenic diet or elevated BHB concentration. I think it would also be interesting to look at both of those effects in rats, as we had the surprising data from the young female rats.

These results improve our understanding of the interplay between metabolism and cognition. Additionally, future research will be able to investigate precise benefits that the ketogenic diet may have in treating and preventing symptoms of diseases of neurodegeneration. Finally, with the basic framework relating to the cognitive effects of the diet being laid by this and prior research, future researchers will be able to discover the precise steps and mechanisms behind these observed effects.

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