INTERMITTENT FASTING MODULATES SYNAPTIC PLASTICITY IN THE MEDIAL PERFORANT PATH OF THE DENTATE GYRUS IN ADULT MALE RATS

By

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ABSTRACT

Intermittent fasting (IF) induces morphological, physiological, and neuronal changes through the process of intermittent metabolic switching (IMS) between glucose and ketone-based metabolism. The dentate gyrus (DG) of the hippocampus, an area of the brain associated with learning and memory, is especially affected by the effects of fasting via the mechanistic signalling of brain derived neurotrophic factors (BDNF) which are thought to play a role in enhancing synaptic plasticity, synaptogenesis, and neurogenesis. The present study sought to determine if IF modulates synaptic plasticity in the medial perforant path (MPP) of the DG, a pathway thought to contribute to the function of long-term spatial memory, in adult male rats, measured by changes in the ability to elicit sustained long-term potentiation (LTP). Our research shows that intermittent fasting does induce sustained LTP in MPP for food restricted (FR) animals. This research provides evidence for the effects of IF on MPP synaptic plasticity and sets the stage for future research involved in determining the mechanisms involved.

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ABBREVIATIONS

2-AG	2-arachidonoylglycerol
AcAc	acetoacetate
ACSF	artificial cerebrospinal fluid
ADF	alternate day fasting
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	5' adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BIC	bicuculine methiodide
BHB	β-hydroxybutyrate
BDNF	brain derived neurotrophic factors
CA	cornu Ammonis
CaMKII	calmodulin-dependent protein kinase II
CRP	C-reactive proteins
DG	dentate gyrus
EC	entorhinal cortex
E-LTP	early long-term potentiation
ERK	extracellular signal-regulated kinase
fEPSP	field Excitatory Post Synaptic Potential
FFA	free fatty acids
FR	food restricted
GABA	neurotransmitter γ-aminobutyric acid
$GABA_AR$	γ-aminobutyric-acid-A Receptors
G-to-K	glucose to ketone
HFS	high frequency stimulation
IF	intermittent fasting
I/O	input/output
K-to-G	ketone to glucose
IMS	intermittent metabolic switch(ing)
IL-6	interleukin 6
LEC	lateral entorhinal cortex
L-LTP	late Long-term Potentiation
LPP	lateral perforant pathway
LTP	long-term potentiation
MCT	monocarboxylic acid transporters
MEC	medial entorhinal cortex
MF	mossy fibers
mGluR	metabotropic glutamate receptors
MPP	Medial perforant path
mTOR	mammalian target of rapamycin
nAcR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptors
NT-3	neutrophin-3
PGC1α	peroxisome proliferator-activated receptor γ coactivator 1α
PND	post-natal day
PP_{50}	paired pulse 50ms
PP_{100}	paired pulse 100ms
PPD	paired pulse depression
PPF	paired pulse facilitation
PPR	paired pulse ratio
PTP	post tetanic potentiation
SC	Schaffer collaterals
SEM	standard error of the mean
STP	short-term potentiation
TCA	tricarboxylic acid
TNFα	tumor necrosis factor α
TRF	time restricted feeding
VGCC	voltage gated calcium channel

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1. INTRODUCTION

1.0 Fasting

Fasting is defined as the process of abstaining from or restricting the consumption of food and/or liquid for an extended period of time.

1.1.0 History of Fasting

In today's society, the idea of fasting, in any capacity, is not common practice outside of religion, athletics, and medicine. Modern society has developed to the point whereby food scarcity is no longer in issue in 1st world countries. Most people, without thought, have developed the habit of consuming a minimum of three meals a day (Bramble & Lieberman, 2004; Mattson, 2014; Mattson, Moehl, Ghena, Schmaedick, & Cheng, 2018). However, from an evolutionary standpoint, both predatory and prey mammals, including humans, often went for long periods of time without food. Natural selection favoured animals that could sustain high functioning brains and nervous systems to solve problems and acquire food while experiencing hunger and food scarcity (Mattson, 2015). Ancestral humans functioned in this manner. Spacing between meals was generally no less than a day for most predators (Gervasi et al., 2012; Mattson, 2015). While fasting is no longer a habitual or forced practice for most humans, it is still used in many circumstantial settings (Patterson & Sears, 2017).

1.1.1 General Effects of Fasting

The main, and biggest effect of transitioning from a satiated to fasted state involves the depletion of liver glycogen stores and the production of ketone bodies, converted from free fatty acids (FFA). This process, is considered part of the *intermittent metabolic switch* (IMS) or the G-

to-K/K-to-G switch (Anton et al., 2018; Mattson et al., 2018). Though the premise of this paper covers the effects IF on synaptic plasticity, this section will first provide a brief background on how IF impacts other physiological and molecular functions, as they too, play a role in how IF influences brain health and function.

In general, while most of the forthcoming effects also have been proven to similarly affect humans, we will focus on the larger body of research concerning animal studies. Fasting has been found to reduce visceral fat levels while allowing for lean body mass retention (Gotthardt et al., 2016; Mattson, Longo, & Harvie, 2017; Varady, Roohk, Loe, McEvoy-Hein, & Hellerstein, 2007), improve glucose metabolism and insulin sensitivity while reducing blood glucose and insulin (Arum et al., 2014; Duan, Guo, Jiang, Ware, & Mattson, 2003), decrease levels of circulating leptin and increase levels of circulating adiponectin, associated with increasing FFA oxidation, a key player to ketone metabolism (Duan et al., 2003; Varady et al., 2007; Wan et al., 2010), and reduce resting heart rate and blood pressure during the fasted periods, associated with improved cardiovascular health (Mager et al., 2006; Wan et al., 2010) (Figure 1.1). Additionally, IF has been found to reduce total cholesterol, associated with cardiovascular risk markers such as C-reactive proteins (CRP), tumor necrosis factor α (TNFα), and interleukin 6 (IL-6), all three of which are associated with inflammation (Mattson et al., 2017).

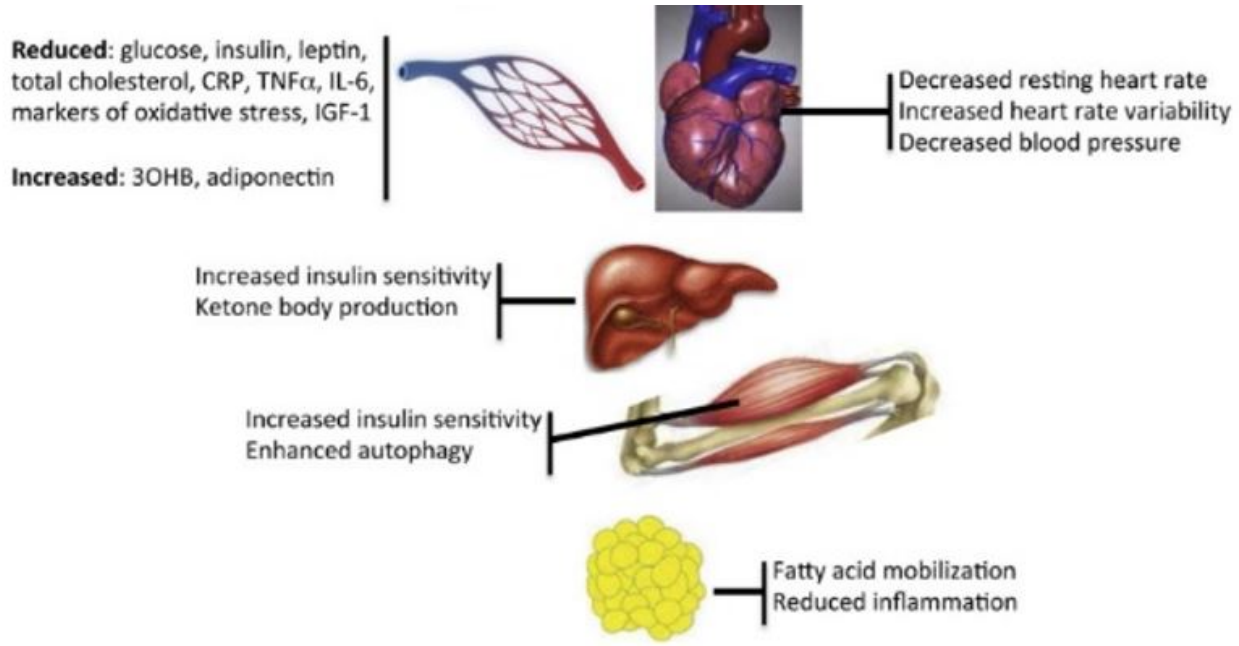

Figure 1.1 - Overall Effects of Intermittent Fasting on the Body. The metabolic switch of glucose to ketone metabolism induced by intermittent fasting has been found to have a number of physiological and metabolic influences, some of which are shown in the above diagram. Modified, with permission, from Mattson et al. (2017).

1.1.2 Fasting and the Brain

With regards to IF's effects on the brain, its biggest influence is on the upregulation of brain derived neurotrophic factors (BDNF) (Lu, Christian, & Lu, 2008; Mattson, Maudsley, & Martin, 2004; Mattson et al., 2018). Upon the occurrence of the G-to-K IMS, the brain no longer uses glucose as its primary source of fuel. Though the brain preferentially uses glucose as its main form of energy, it is fully capable of efficiently using other forms of energy such as ketone bodies (Courchesne-Loyer et al., 2017). The ketone β-hydroxybutyrate (BHB), when transported into neuronal cells in the brain, can be used as a form of energy to be converted into adenosine triphosphate (ATP) (Camandola & Mattson, 2017; Courchesne-Loyer et al., 2017), but also to activate signalling pathways to enhance the transcription of BDNF, a key mediator in synaptic plasticity (Figure 1.2). Additionally, IF has been shown to activate stress-response cascades and autophagy during fasted states via depletion of blood glucose, leading to the

activation of AMP kinases (AMPK) and simultaneously, the reduction of mammalian target of rapamycin (mTOR), which also upregulates AMPK signalling via a positive feedback loop, all to allow for the recycling of cellular machinery and DNA repair (Mattson et al., 2018). With regards to brain function, IF has been found to induce protein synthesis and mitochondrial biogenesis in neurons during the re-feed state, which can lead to neurogenesis, or the creation of new neurons, in the hippocampus (Anton et al., 2018). Mitochondrial biogenesis, upregulated by BDNF, is also upregulated by the transcription factor peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α). Additionally, proliferator-activated receptor γ coactivator 1α, activated from increased excitatory post-synaptic Ca^{2+} influx, also plays essential role in maintenance of dendritic spines of hippocampal dentate gyrus (DG) cells through its own signalling cascades and also through its co-activation of BDNF (Cheng et al., 2012). Brain derived neurotrophic factors and PGC1α have positive feedback signalling loop whereby they both upregulate each other and together, enhance and strengthen the neurons' cellular machinery in hippocampal granule cells (Mattson et al., 2018).

Previous research has found that both rats and mice that underwent an IF protocol showed enhanced long-term potentiation (LTP) in the hippocampus (Farmer et al., 2004; O'Callaghan, Ohle, & Kelly, 2007; Talani et al., 2015; van Praag et al., 2002), a form of synaptic plasticity to be discussed later in the introduction. Increased BDNF levels, shown to lead to an increase in dendritic spine density in hippocampal dentate granule neurons of both mice (Stranahan et al., 2009) and rats (Talani et al., 2015), as a result of IF, is thought to be a contributing factor as to how IF leads to enhanced synaptic plasticity.

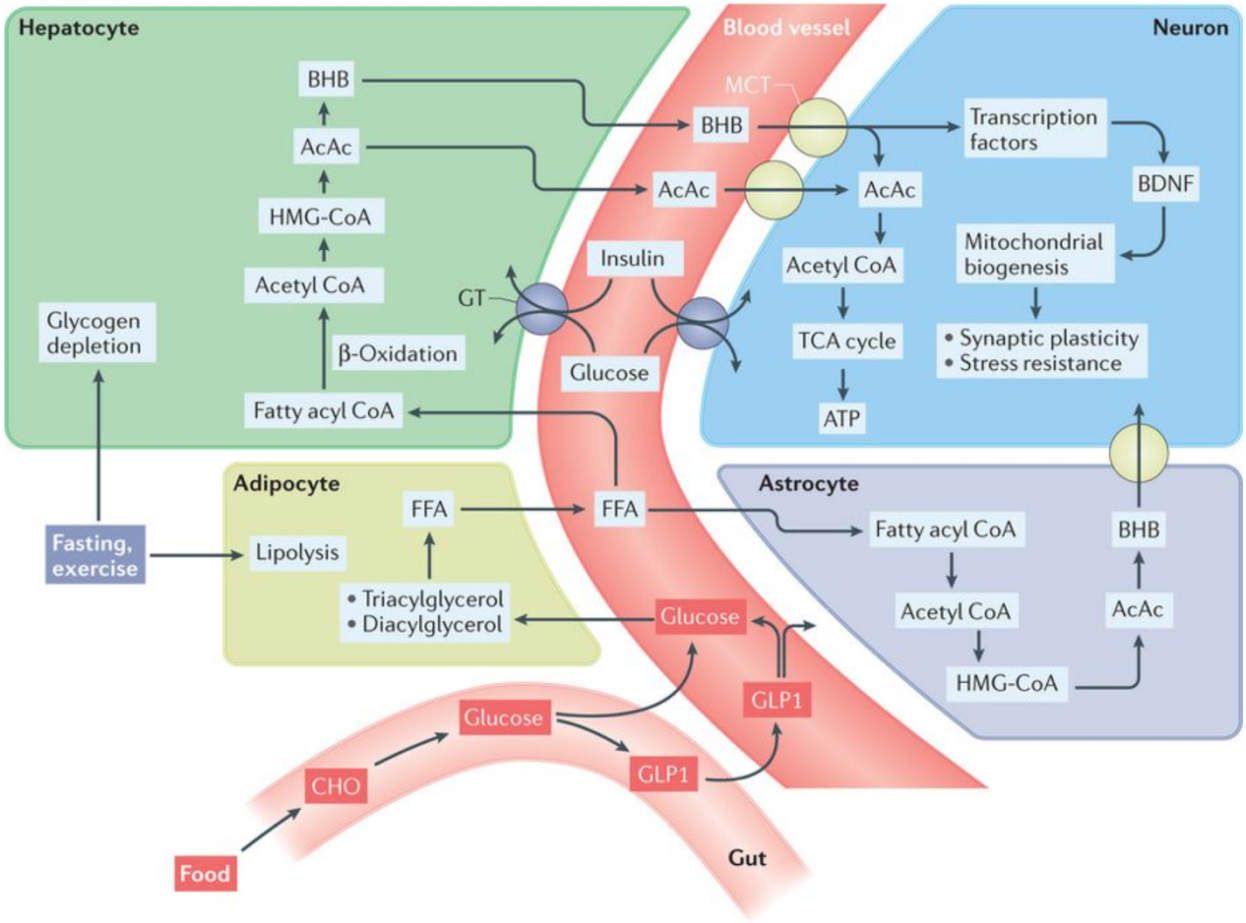

Figure 1.2 - Overview of Biochemical Pathways Involved in the IMS from Body to Brain. Both fasting and exercise act to initiate the depletion of glycogen in hepatic liver and the lipolysis of tri-/di-acyclglycerols into free fatty acids (FFA) in visceral adipocytes, which are released into the bloodstream. Free fatty acids are converted into ketones acetoacetate (AcAc) and β-hydroxybutyrate (BHB) in both hepatocytes and astrocytes in the brain, whereby they are transported into neurons through monocarboxylic acid transporters (MCTs). Ketones in neurons can then be converted into energy in the form of adenosine triphosphate (ATP) via the tricarboxylic acid (TCA) cycle in the neuronal mitochondria. Most importantly, BHB can be imported into neurons to upregulate brain derived neurotrophic factor (BDNF) transcription, which indirectly promotes synaptic plasticity. Adapted from Mattson et al. (2018).

1.1.3 Fasting Models

The amount of time that defines a fast is a commonly debated topic in the literature. There are mainly models of fasting that are fundamentally different, but in general, there are two broad categories being: pure fasting, and intermittent fasting (IF). The main differentiation between these two categories is with their timing. Generally, pure fasting is regarded to as fasts that last upwards of several consecutive days without food, and sometimes without water, too. Intermittent fasting on the other hand, is an umbrella term for a variety of ways to restrict caloric consumption for a certain period of time (Patterson & Sears, 2017). Generally speaking, most IF styles of eating are considered when the fasts last for no longer than 24 hours at a time.

It should be noted that while fasting is often colloquially referred to as a form of dieting, it is not a meal plan. Fasting does not focus on the composition of macro-nutrients that are consumed by the individual on a daily basis but rather period of time between meals.

1.1.3.1 Intermittent fasting

As mentioned, there are many styles of food restriction that fit under the IF umbrella term and while they all initiate similar, and/or the same, metabolic process, the timing of their paradigms, differs. The two most common forms of IF will be discussed here. One of the most commonly used forms of fasting used in current western cultures, outside of religion, is restricting food intake to a 2-10 hour daily period, also referred to as time restricted feeding (TRF) (Figure 1.3) (Chaix, Zarrinpar, Miu, & Panda, 2014; Mattson et al., 2017; Patterson & Sears, 2017). Time restricted feeding has been successfully implemented in a number of different human trials and rodent models and has been shown to elicit positive health outcomes despite the variable effect of potential weight loss in these models (Patterson & Sears, 2017). With regards to rodent models, TRF has been found to not only be an effective tool as an intervention strategy for weight loss in

obese male rodents, but also postmenopausal female rodents, indicating that TRF may be a translational intervention paradigm for both men and women (Chung et al., 2016; Patterson & Sears, 2017).

Another popular form of IF used both in clinical and animal trials is alternate-day fasting (ADF), whereby an individual will have *ad libitum* access to food and water on the feeding day, but will water fast on the alternate day. Alternate day fasting studies in rodents has been found to be similarly effective to TRF for reducing obesity-associated weight, improving insulin sensitivity, and enhancing mechanistic processes associated with cell proliferation and neurocognitive function (Mattson et al., 2017; Patterson & Sears, 2017). Nonetheless, a number of studies have found that ADF did not substantially improve metabolic risk factors (Patterson & Sears, 2017) in humans of non-obese weight. Moreover, some research has found that ADF, when compared to TRF, does not positively impact mood or perceived energy levels in human adults as much (Antoni, Johnston, Collins, & Robertson, 2016; Appleton & Baker, 2015; Heilbronn, Smith, Martin, Anton, & Ravussin, 2005).

Overall, there is currently more research on IF models using animal models. Meta-analyses from the literature has shown that IF in rodent models, and early human clinical trials, has positive impacts on physiological aspects of health in the treating diseased populations and also physiological and molecular markers of longevity in healthy subjects (Horne, Muhlestein, & Anderson, 2015; Patterson & Sears, 2017).

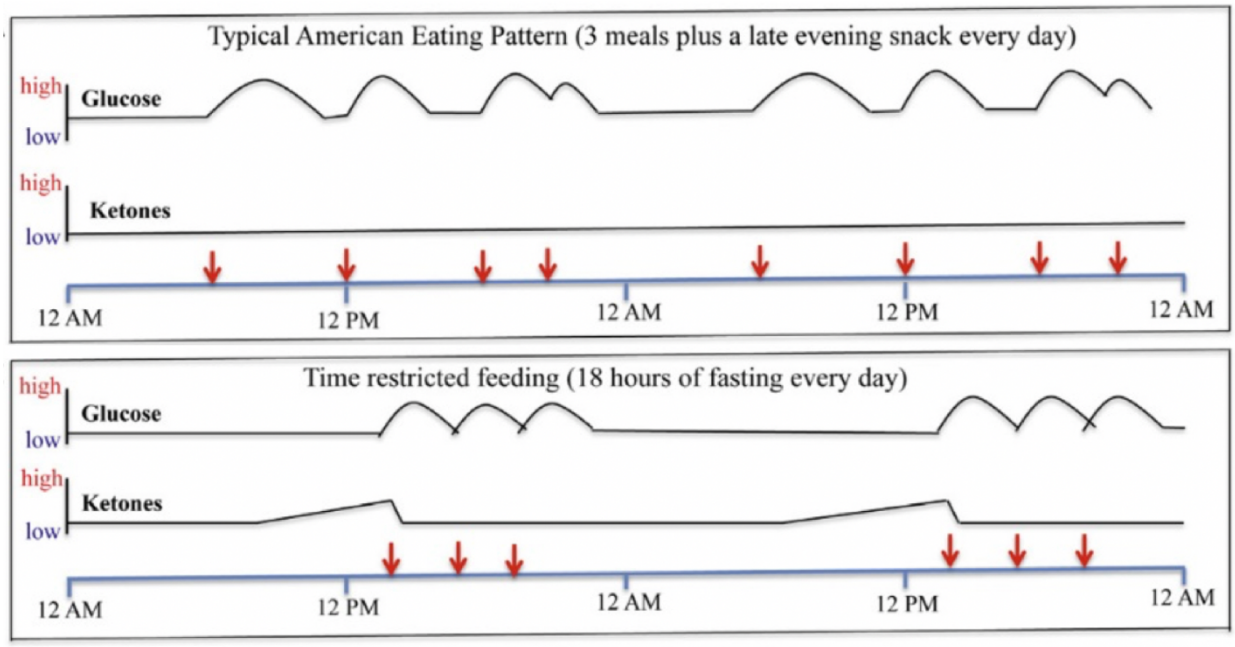

Figure 1.3 - Depiction of Inverse Ketone-Glucose relationship and how time restricted feeding (TRF or FR), a form of intermittent fasting, elevates blood ketone levels. Typically, frequent meal eating patterns (*above*) leads to decreases in blood glucose regulation and decreased insulin sensitivity while TRF (*below)* has the opposite effect, and leads to elevated blood ketone levels which have a number of beneficial downstream effects on the brain and body. Adapted and modified from Mattson et al. (2017).

1.2 The Hippocampus

1.2.1 Hippocampus History and Function

The hippocampus (Figure 1.4) is a medial temporal lobe limbic structure in the brain widely known to be associated with learning and memory (Bliss & Collingridge, 1993; Corkin, 1968; Eichenbaum, 2004; Teyler & DiScenna, 1985). Before anatomical and functional discoveries were made, scientific curiosity of the hippocampus stemmed as far back as 1587 when the structure was given its name by the Greek anatomist, Julius Caesar Aranzi, for its resemblance to a sea horse of Greek mythology (Andersen, Morris, Amaral, Bliss, & O' Keefe, 2009). The discovery of the functional role of the hippocampus arose only more recently in the 1900s, from the famous case of Henry Molaison who had a bilateral medial temporal lobe removal resulting in anterograde

amnesia (Scoville & Milner, 1957). This finding paved the way for further research looking at hippocampal function with regards to disease and health.

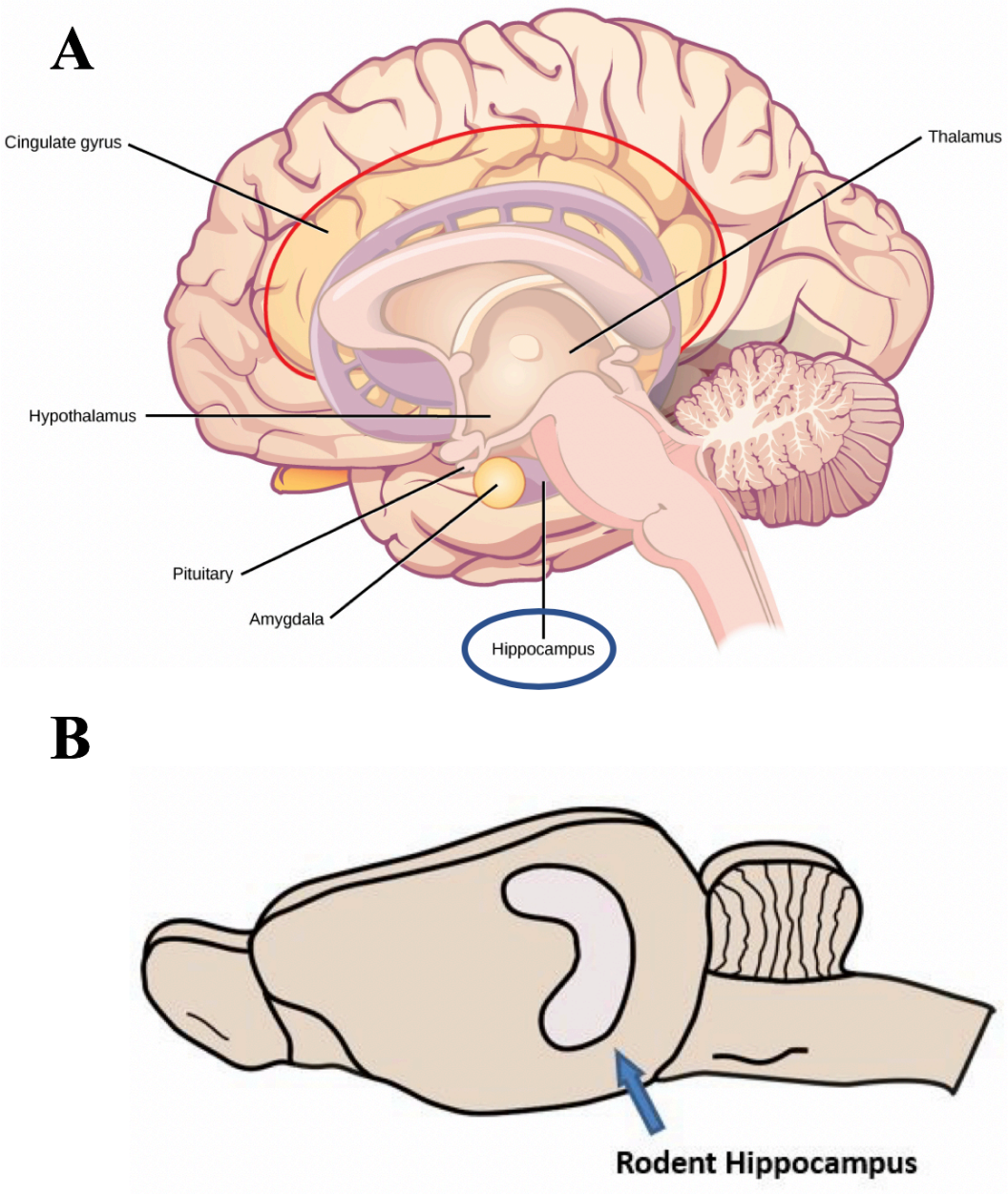

Figure 1.4 - Anatomical comparison of Human and Rat hippocampal placement. (**A**) Depiction of human hippocampal placement relative to other mid brain structures. Source courtesy of CFCF via wikicommons free use copyright http://bit.do/Hippocampus. (**B**) Depiction of rodent hippocampal placement.

1.2.2 The Hippocampal Formation and Trisynaptic Circuit

The stereotypic layout of the hippocampal formation, in rats, resembles a half circle or banana shape that extends dorsoventrally (septotemporal axis) from the septal nuclei, up, over, and behind the thalamus, to the start of the medial temporal lobe (Andersen et al., 2009). The defining characteristic of the hippocampal formation is its primarily unidirectional arrangement of pathways that connects its substructures. These substructures are cytoarchitecturally different, and generally categorized as the hippocampus proper, also referred to as the Ammon's horn (Andersen et al., 2009) which is divided up into the *cornu Ammonis* (CA) regions 1 through 4 (Andersen et al., 2009; Bayer, 1980; Lavenex, Banta Lavenex, & Amaral, 2006), the dentate gyrus (DG), subiculum, presubiculum, parasubiculum, and entorhinal cortex (EC) (Andersen et al., 2009). These pathways, extending from the EC to the DG, then through CA regions to subiculum, and ultimately back to the EC, are strongly interconnected and function together to process and integrate sensory/spatial information for further downstream cortical structures including the EC and prefrontal cortex (Agster & Burwell, 2009). This completed pathway can be seen as an electrical circuit, which is why, altogether, it is referred to as the trisynaptic circuit (Figure 1.5).

The first stage of the hippocampal circuit to be considered is the EC. The EC, placed between the cerebral cortex and the hippocampus, projects axons of Superficial neurons into the DG via the perforant pathway (Lømo, 1971). The EC itself is divided into 6 layers, unlike 3 in the hippocampus, and is subdivided into the Medial (MEC) and lateral (LEC) entorhinal cortices (Andersen et al., 2009). The majority of the perforant axons synapse DG granule cell spiny dendrites, in an *en passant* fashion, which in turn project their axons, referred to as the mossy fibers (MF) to the CA3 pyramidal neurons (Andersen et al., 2009). CA3 neurons project their axons, Schaffer collaterals, to the upstream CA1 pyramidal neurons, which then project their

neurons to the subiculum. The Subiculum sends direct projections back to the EC, completing the circuit, but also have projections that innervate the presubiculum and parasubiculum. Additionally, neurons in the subiculum and CA1 also send direct projections to the deep layers of the entorhinal cortex (Andersen et al., 2009).

1.2.3 The Dentate Gyrus and the Perforant Pathway

The DG is a hippocampal structure that is unique to mammals (Treves, Tashiro, Witter, & Moser, 2008). The DG has been found to be a key structure in the role of several major processes. Studies of the DG have found that it functions in playing a role in processing spatial patterns and integrating sensory input from sensory processing areas (Kesner, 2013; Treves et al., 2008). Therefore, it is likely that the DG is a large contributor to the function of long-term spatial memory (Hunsaker, Mooy, Swift, & Kesner, 2007). The DG is made of 3 layers being the molecular layer, the granule cell layer, and the polymorphic cell layer, from top to bottom, respectively (Amaral, Scharfman, & Lavenex, 2007).

The source for excitatory input into the DG is the perforant pathway, which can be subdivided into the medial and lateral perforant paths (MPP & LPP) based on the positioning of synapses on the granule cells in the molecular layer, as well as the origin of projections from the EC (Witter, 2007). This dichotomous distinction of there being two pathways is well supported in the literature by histochemical, functional, and plasticity studies (Amaral et al., 2007; Bramham, Errington, & Bliss, 1988; McNaughton, 1980; Witter, 2007). The perforant pathway itself arises mostly from layer II stellate cells of the EC (Steward & Scoville, 1976; Witter, 2003). In particular, the MPP afferents originate in the MEC and exclusively synapse the middle third dentate gyrus while the LPP afferents originate in the LEC and exclusively innervate the distal third (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Petersen et al., 2013; Steward & Scoville, 1976).

Moreover, while there is debate in the literature on whether the different pathways have different functional implications, there is some evidence to suggest that the MPP plays more of a role in conveying spatial information to the DG while the LPP conveys non-spatial information such as olfactory and auditory information (Hunsaker et al., 2007). Additionally, while the majority of perforant synapses are considered excitatory with glutamate being the primary neurotransmitter of release, there are a small fraction of terminals that synapse onto interneurons of the DG molecular layer that trigger the release of inhibitory neurotransmitter γ-aminobutyric acid (GABA), which will be discussed more following.

One differentiation, when it comes to pharmacological and physiological properties of these pathways is that receptor distribution and function acts differently within these pathways. Field excitatory post synaptic potential (fEPSPs) tend to be more greatly depressed in the MPP when agnostic treatments for gamma-aminobutyric acid A receptors ($GABA_AR$) are applied whereas LPP fEPSPs tend to be more greatly depressed when group III metabotropic glutamate receptor (mGluR) antagonists are used (Koerner & Cotman, 1981; Lanthorn & Cotman, 1981). Additionally, cholinergic agonists and N-methyl-D-aspartate receptor (NMDAR) antagonists have been shown to only depress MPP fEPSPs, while not having an effect on LPP fEPSPs (Dahl, Burgard, & Sarvey, 1990; Kahle & Cotman, 1989).

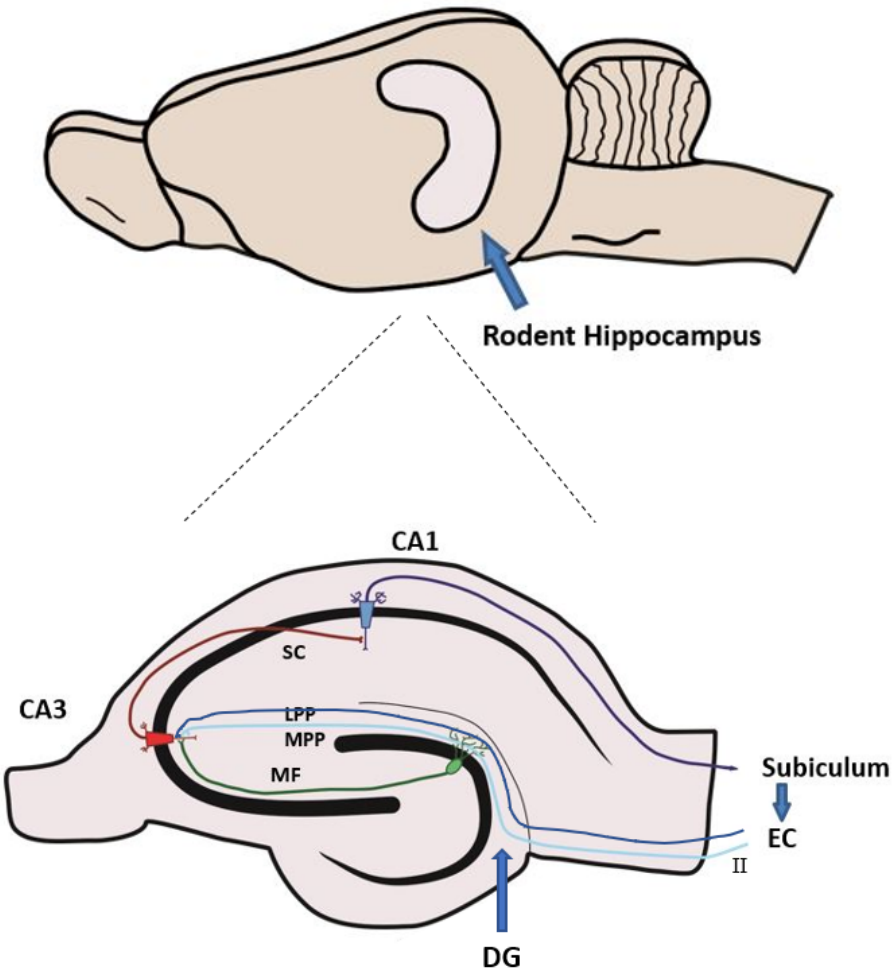

Figure 1.5 - Schematic depiction of hippocampal structure and function from a tissue to synaptic level. Simplified sagittal illustration of the rodent brain (*top)*, showing the relevant positioning of one hippocampi relative to the rest of the brain. Simplified illustration of a cross-sectional slice of the hippocampus showing the tri-synaptic circuitry involved in communicating information to and from the Entorhinal cortex (EC) (*bottom*). The primary route of communication is via the perforant pathway, with both the medial perforant pathway (MPP) and Lateral perforant pathway (LPP) originating out of layer II of the EC, projecting to both the cornu ammonis 3 (CA3) pyramidal neurons, directly, and to the granule cells of the dentate gyrus, in an en passant fashion. These granule cells also project to the CA3 pyramidal neurons via their axons, the mossy fibers (MF). The CA3 neurons project to the cornu ammonis 1 (CA1) pyramidal neurons via their Schaffer collateral (SC) axons. Lastly, the CA1 pyramidal neurons project efferently from the hippocampus to the subiculum, which projects to the EC.

1.3. Synaptic Plasticity

The idea of synaptic plasticity in the hippocampus, that synapses have the ability to change and adapt within complex neural circuits to mediate the storage of information during learning, was first investigated by the famous neuroscientists Cajal and Hebb (Cajal, 1893; Hebb, 1949). Later, the process whereby repeated electrochemical stimulation, or a lack thereof, alters, both pharmacological and physiological properties of synapse, both pre- and postsynaptically, over time, was more appropriately understood as a form of plasticity (Hughes, 2017; Lomo, 1966).

From a basic standpoint, neurons within the nervous system communicate primarily through electrochemical means. Each neuron that fires an action potential, or an electrical potential, propagates its electrical signal down its axons into its boutons whereby chemical cascades are then activated. Upon neurotransmitter release into the synaptic cleft, these chemical messengers can interact and bind to receptors on the post synaptic membrane of another neuron's dendrite bouton (Purves, Augustine, & Fitzpatrick, 2012).

From a more detailed perspective of what happens in most pre-synaptic membranes in the transition from electrical to chemical processes, the propagation of an action potential down a neuron's axon into a bouton activates and opens voltage gated Ca^{2+} channels (VGCC) which triggers the cascade for vesicular neurotransmitter release into the synaptic cleft (Andersen et al., 2009; Purves et al., 2012; Zucker & Regehr, 2002).

The process of plasticity can be separated in to early and late types, and can be expressed in both pre-synaptic and post synaptic terminals (Castillo, 2012). The types of strengthening plasticity that occurs over time follows in the order of facilitation, also referred to as paired pulse facilitation (PPF), in the span of a few milliseconds, augmentation which occurs over a few seconds, and then potentiation which occurs of the time span of 10 seconds to minutes to hours

(Purves et al., 2012). These forms of facilitation and potentiation can be directly evoked after eliciting high frequency stimulations to the tissue. Post-tetanic potentiation (PTP), short-term plasticity (STP), and then long-term potentiation (LTP) also differ not only by the mechanisms which induce them, but by time, ranging from 1 minute, to several minutes, to 1 hour or more, respectively (Purves et al., 2012). In general, while these forms of plasticity are related in the sense that they arise from prolonged elevation of presynaptic calcium, the molecular mechanisms that assist in these processes are still somewhat unknown (Purves et al., 2012).

Long-term potentiation, the main focus of this paper, can be divided in to early-LTP (E-LTP) and late-LTP (L-LTP) (Figure 1.6), which are primarily either protein kinase dependent or protein synthesis dependent, respectively (Lu et al., 2008).

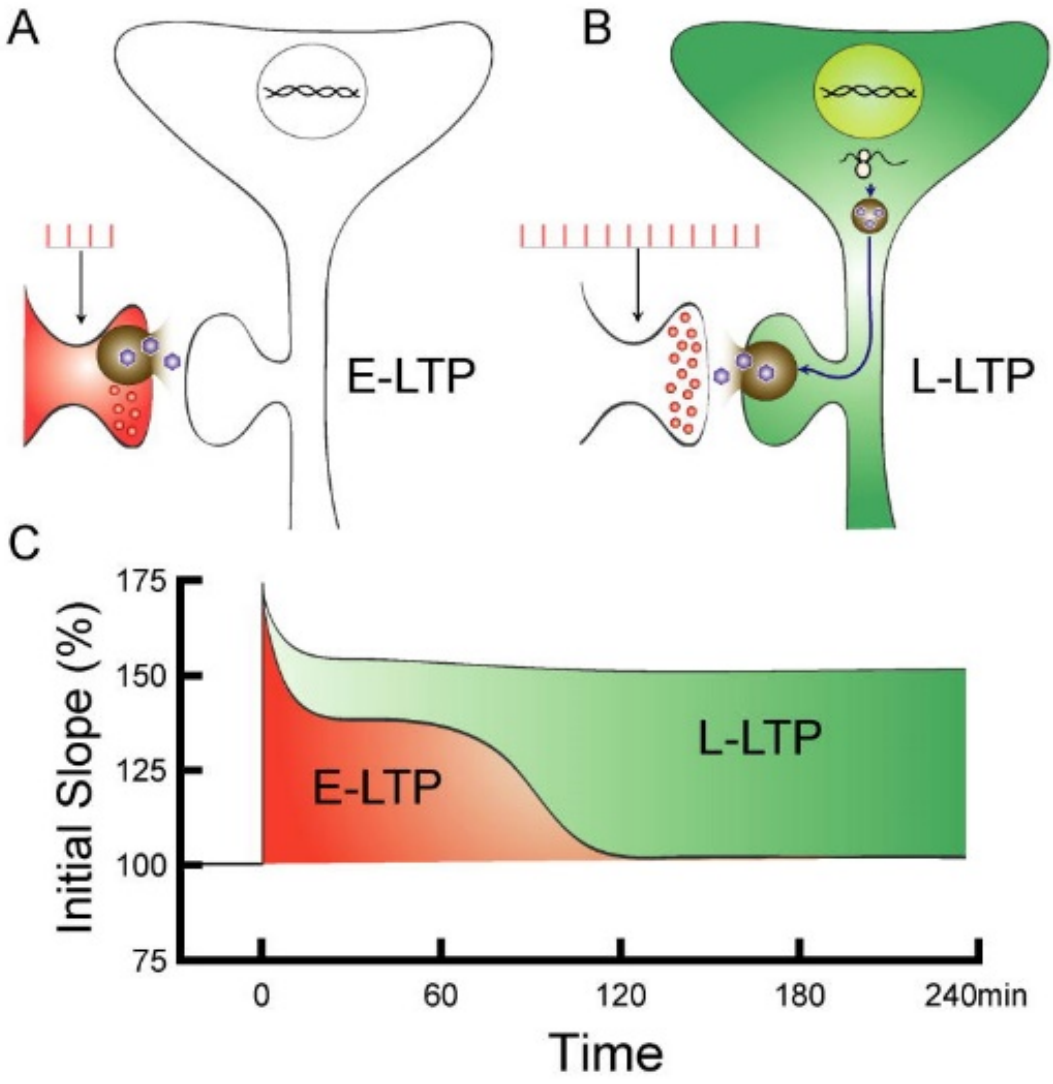

Figure 1.6 - Depiction of the Influence of BDNF on Early and Late Long-term Potentiation. (**A**) Depiction of the role of brain derived neurotrophic factor (BDNF) in early long-term potentiation (E-LTP) acting as a presynaptic modulator for potentiation involving protein kinase function. (**B**) Depiction of BDNF acting postsynaptically in late long-term potentiation (L-LTP) via protein synthesis mechanisms. Source adapted from Lu et al. (2008).

1.3.1 Mechanistic influences of MPP LTP

The focus of this paper is on the MPP. The mechanisms underlying LTP in the MPP have been more thoroughly studied than in the LPP. In general, the most well-known form of LTP in the MPP is characterized as NMDAR-dependent LTP, whereby presynaptic glutamate release induces activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, the

unblocking of Mg^{2+} ion blockers in NMDA channels, and the influx of calcium which activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) leading to the upregulation of AMPA receptors to the postsynaptic membrane (Bliss & Collingridge, 1993; Bramham, 2007). Additionally, however, research has also shown that MPP LTP is co-activated by nicotinic acetylcholine receptors (nAcR), and VGCCs (Nashmi et al., 2007). In terms of plasticity regulation, LTP is primarily regulated by GABAergic facilitation of DG interneurons (Bramham, 2007) and group II mGluR autoreceptors, unlike group I and III in the LPP (Macek, Winder, Gereau, Ladd, & Conn, 1996; Matsuo, Reijmers, & Mayford, 2008).

In terms of neurotrophic factors, BDNF in the MPP, as opposed to neutrophin-3 (NT-3) in the LPP, has been shown to support synaptic LTP in DG granule cells in a process known as BDNF-LTP, acting in the transition of E-LTP to L-LTP (Lu et al., 2008).

With regards to the endocannabinoid system, research has shown the endocannabinoid 2-Arachidonoylglycerol (2-AG) and its CB_1 receptor play little to no role in the contribution of sustained LTP in the MPP region while the opposite is found in the LPP (Wang et al., 2016) and CA1 (Talani et al., 2015) regions. Mechanistic reasons for this are still widely unknown.

1.3 The Present Study

The present study sought to determine if IF modulates synaptic plasticity in the medial perforant path (MPP) of the DG, a pathway thought to contribute to the function of long-term spatial memory, in adult male rats, measured by changes in the ability to elicit sustained long-term potentiation (LTP). We hypothesized that we would see enhanced and sustained LTP in the MPP of adult rats since IF has been previously shown to upregulate dendritic spine densities of DG granule cells via BDNF signalling.

2. METHODS

2.1 Animal Acquisition

2.1.1 Animal Housing and Acclimation

All experimental procedures involving animals were carried out in accordance with the Institutional Animal Care Committee (AUP #2018-017) at the University of Victoria and held to the standards set by the Canadian Council of Animal Care. Sexually mature male Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, PQ, Canada). The rooms were maintained on a 12-hour light-dark cycle with constant humidity and temperature (22°C).

Upon arrival, animals were housed in clear polycarbonate cages (46x24x20 cm) with a single red polycarbonate shelter hut. Initially, animals were caged in pairs or threes and allowed to acclimate to the new environment from post-natal day (PND) 30-40 to PND 50-60, for 10-30 days, respectively. Halfway through their acclimation period, animals were separated and caged individually. Nearing the end of acclimation, animals were randomly assigned to a control condition or to the IF protocol. During the acclimation period, all animals were given *ad libitum* access to water and chow (Lab Diets 5001, Labdiets, Richmond, IN, USA) at all times. During the entirety of this project, all animals were given *ad libitum* access to water.

2.1.2 Fasting Protocol and Control Animals

The fasting protocol started after the acclimatization period at PND 50-60 and lasted for 3-4 weeks, where animals were then taken for electrophysiological experiments between the ages of PND 70-90. Intermittently fasted animals were given a 2-hour eating window during the light cycle (4:00-16:00) and were given *ad libitum* access to chow, in addition to water, during this time.

Control animals were placed in the exact same environment but were given *ad libitum* access to food and 24 hours per day.

During the 3-4 week IF protocol, daily measurements of food restricted (FR) and control rats were taken. Body weight, in addition to food consumption, was measured after the 2-hour eating window for both FR and control animals.

2.2 *in Vitro* Electrophysiology

2.2.1 Slice Preparation

Male offspring were used for *in vitro* electrophysiology between the ages of PNDs 70-90. Rats were deeply anesthetized with inhaled isoflurane (Isoflurane USP, Fresenius Kabi Canada Ltd., Richmond Hill, ON, Canada), then rapidly decapitated after in the absence of the toe or tail-pinch reflexes. The brain was rapidly removed and placed into ice cold, oxygenated (95% O_2/5% CO_2) artificial cerebral spinal fluid (aCSF; 125.0 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 1.3 mM $MgCl_2$, and 10.0 mM dextrose). During this time, the total brain and liver weights were recorded. The brain was dissected to remove unnecessary structures including the olfactory bulbs, the cerebellum and white matter tracts from the brainstem. Hemispheres were separated along the longitudinal fissure and a 30° cut was made along the dorsal side, acting as gluing surface to accommodate for the slight angulation of the trisynaptic circuit during slicing. Both hemispheres were glued (Vetbond, 3M Animal Care Products, St. Paul, MN, USA) in place on the chuck. Transverse hippocampal slices (400 μm) were sliced using a Vibratome Section System 1500 (Ted Pella, Redding, CA, USA) (Figure 2.1). Slices were transferred into an oxygenated incubation chamber aCSF bath heated at 32°C for 10 minutes and then allowed to cool to room temperature. Slices were incubated for 45 minutes before beginning the electrophysiological experiments.

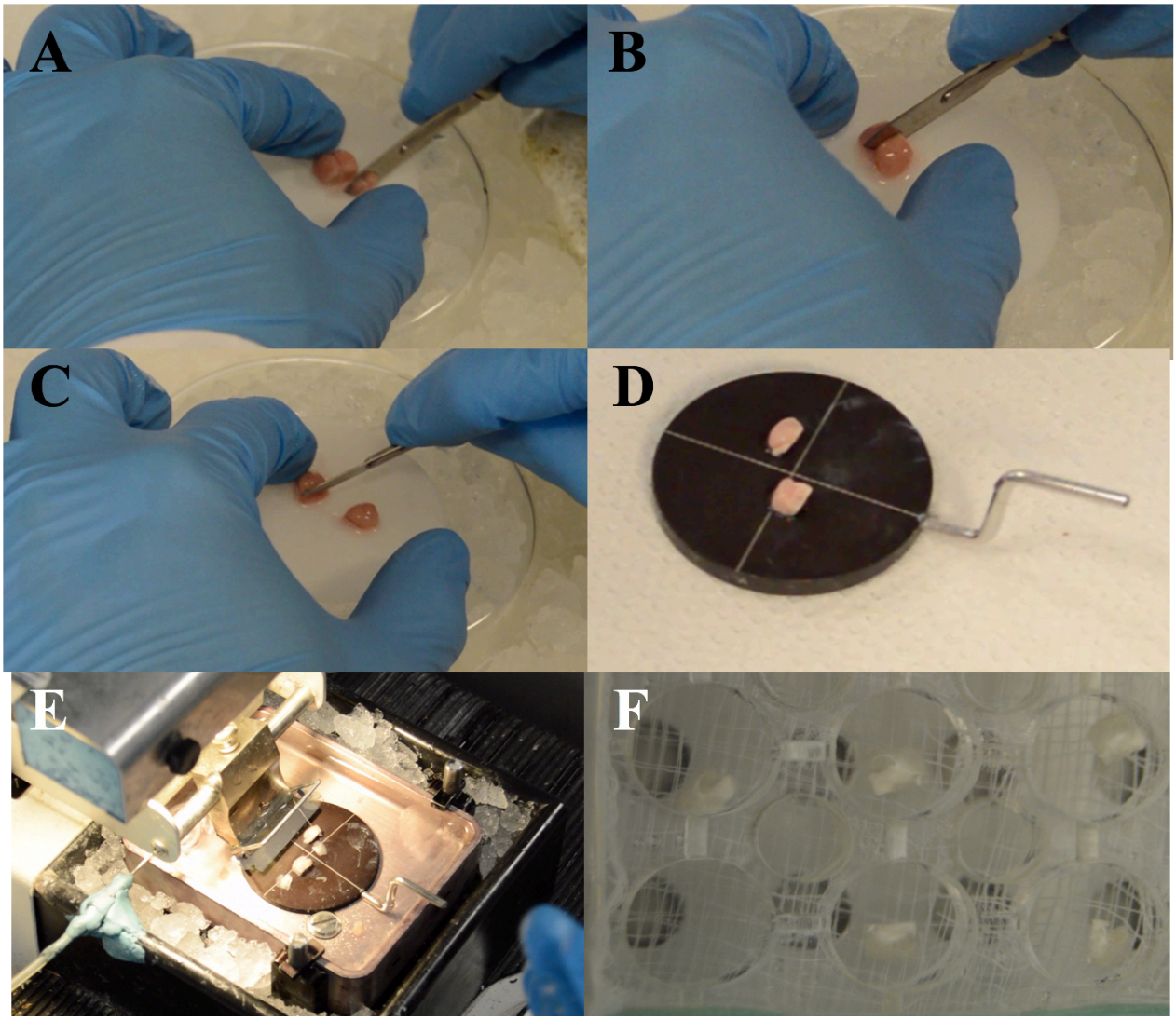

Figure 2.1 – Brain Preparation of Hippocampal Slices. (**A**) Removal of cerebellum and frontal lobes. (**B**) Separation of left and right hemisphere down the longitudinal fissure. (**C**) 30º transverse cut made into the dorsal surface of the brain. (**D**) Hemispheres glued using Vetbond to the mounting disk for the vibratome. (**E**) Vibratome slicing 400µm slice. (**F**) Incubation of hippocampal slices in oxygenated aCSF.

2.2.2 Recordings

Field recordings were collected from slices in an oxygenated aCSF bath with a flow rate of approximately 1.5-2 mL/min. Recordings were collected using an Axon MultiClamp 700B amplifier and Clampex 10.6 software (Molecular Devices, CA, USA). Two Olympus microscopes (BX50W1 and BX51W1) were used to visualize and place electrodes into the MPP (Fig. 2.2). MPP placement was confirmed using the Petersen et al. (2013) current-sink source method. Field EPSPs (fEPSPs) were initially elicited by delivering a 120 μs (0.5 mA) current pulse by a digital stimulus amplifier (Getting Instruments, CA, USA) and a single, concentric bipolar stimulating electrode (FHC, Bowdoin, ME, USA). fEPSPs were recorded using a single glass recording electrode (0.5-1.5 MΩ) filled with aCSF (Figure 2.3).

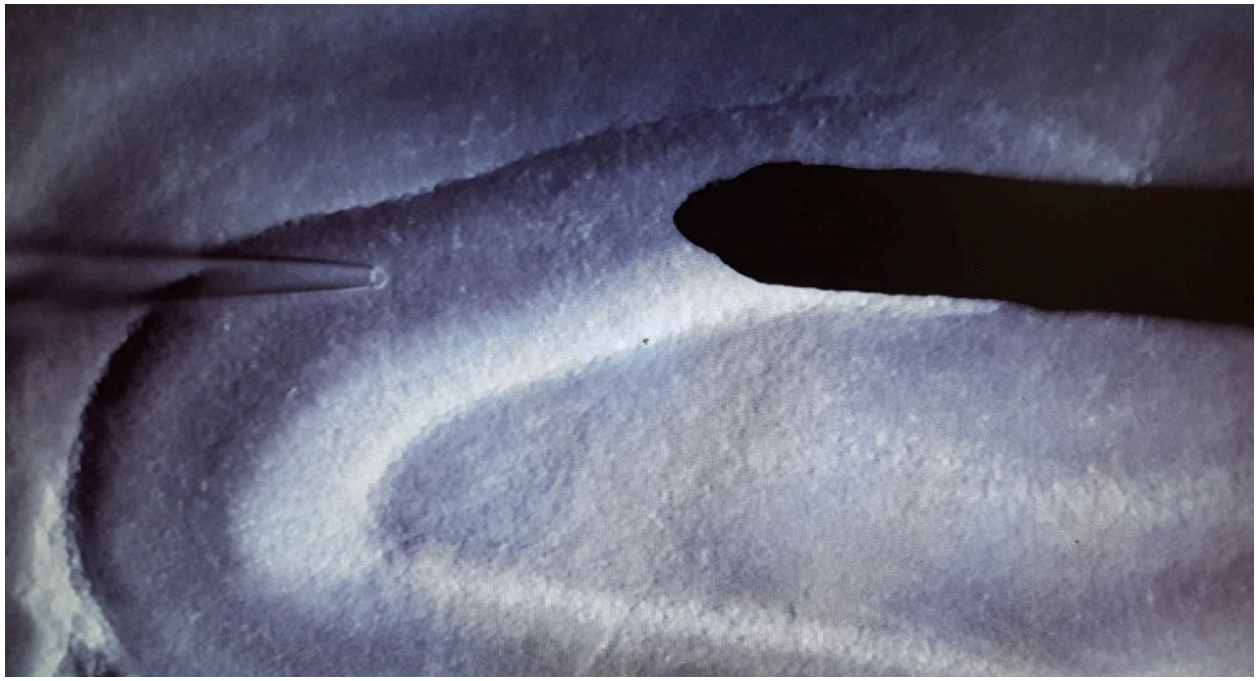

Figure 2.2 – Recordings. In the MPP, the stimulating (*solid black*) and recording (*transparent*) electrodes are placed in the middle third of the molecular layer.

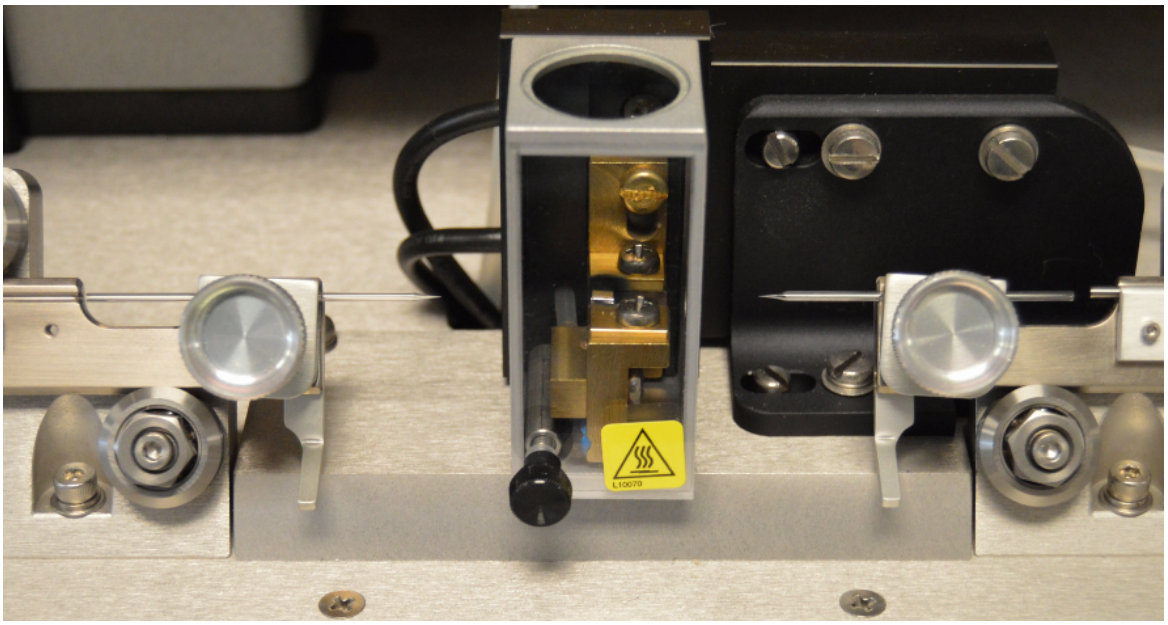

Figure 2.3 – glass electrodes being pulled in Model P-1000 electrode pulling machine. Glass recording electrodes were pulled to create tips able to withstand 0.5-1.5 MΩ tip resistances. Pipette machine used was a Model P-1000 Flaming/Brown Micropipette Puller by Sutter Instruments.

An initial baseline of fEPSPs, evoked every 15 seconds while being held at 0.05mA, was run for approximately 1-5 minutes to insure stability prior to running experimental protocols. Criteria for a stable baseline were based on less than a 10% change in fEPSP slope at any point and an average slope within the range of ±1.5 (figure 2.4). Following this, an input/output (I/O) experiment was conducted with increasing stimulation magnitudes (30-300μs pulse widths at 15s intervals). Paired-pulse (PP_{50} and PP_{100}) experiments were then performed with inter-intervals of 50ms and 100ms (5x; 15s between pairings).

Following I/O and PP experiments, twenty minutes of stable preconditioning recordings were again collected using fEPSPs evoked every 15 seconds but with the stimulation magnitude set to 50% of the maximal response. Following this, potentiation of fEPSPs was induced using an HFS protocol of four trains of 50 pulses at 100Hz 30 seconds apart. Following the HFS protocol, a post-conditioning baseline was ran for 60 minutes.

The $GABA_AR$ antagonist bicuculine methiodide (BIC) (Sigma-Aldrich, Oakville, ON, Canada) was included in the aCSF (20μM) during the initial 20-minute baseline and through the HFS protocol. The BIC then replaced with standard aCSF for the 60-minute decay baseline.

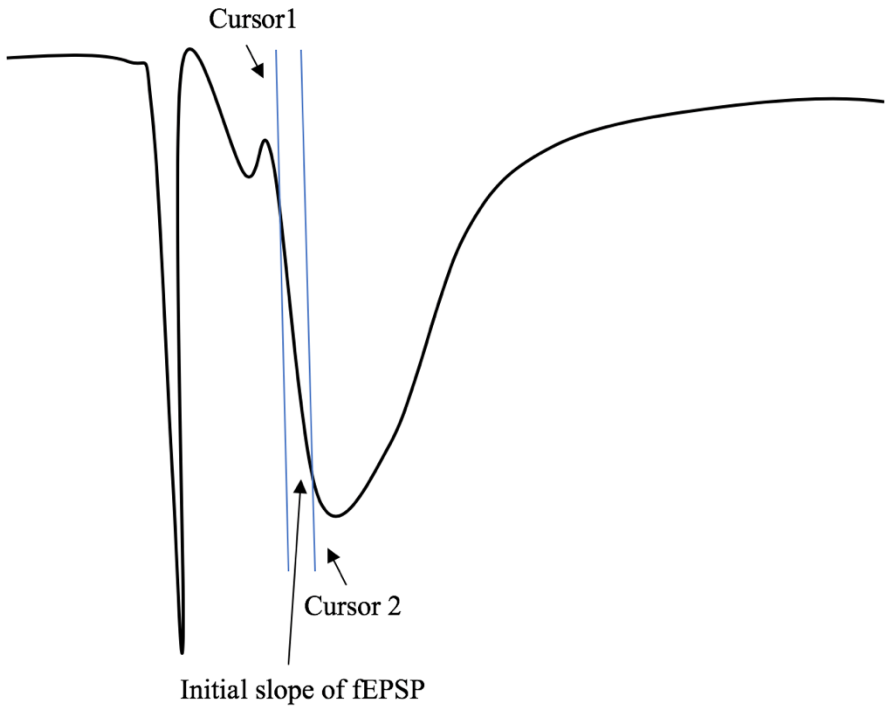

Figure 2.4 - fEPSP Recording. Trace of fEPSP waveform. Slope is calculated based on positioning of the two cursors, which are placed at 10-90% of peak amplitude.

2.3 Data and Statistical Analysis

All electrophysiological data was initially analysed using Axon ClampFit 10.2 software (Molecular Devices, CA, USA). Further data analysis was conducted using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). The Student's t-test was performed for all experimental comparisons, unless otherwise stated, with $p < 0.05$ being the cut off for significance.

2.3.1 Body weight Analysis

Animal body weights and were recorded once daily for the span of the 3-4-week fasting protocol. Individual animal bodyweights were averaged across each experimental condition and between group comparisons were made.

2.3.2 Brain and Liver weight Analysis

Total brain and liver weights were measured and compared between groups as an average of each animal's brain to body weight ratio.

2.3.3 Paired-Pulse Experimental Analysis

Paired-pulse ratios (PPR) were calculated by dividing the slope of the second fEPSP by the first fEPSP. Paired-pulse ratios were taken from the average of their six paired pulse repetitions. A 1.00 ratio indicated no change in fEPSP slopes. A ratio of greater than 1.00 was considered to be PPF and a ratio of less than 1.00, paired pulse depression (PPD).

2.3.4 I/O Experimental Analysis

Input/Output data with varying pulse widths from 0.03ms to 0.3ms, with increasing in intervals of 0.03ms, were analyzed and graphed as fEPSP slope (mV) as a function of pulse width and compared across IF and control conditions.

2.3.5 HFS_{50} Experimental Analysis

All fEPSP slopes were averaged for 1 minute of recording (4 traces) and the post-conditioning fEPSP slopes for all recordings were normalized to the average value of the 20-minute pre-conditioning fEPSP slopes and reported as the average percent change from baseline ± standard error of the mean (SEM). Parameters for data inclusion included the following:

1. Pre-conditioning fEPSP recordings did not exceed a variability of ± 10%.

2. The average percent change of fEPSP slope in the last 5-minutes of post-conditioning recording did not exceed ± 10%.
3. The average slopes of the 20-minute preconditioning baseline and last 5 minutes of the post-conditioning baseline did not exceed ± 1 and ± 2, respectively.

PTP was calculated as the % change of fEPSP slope of the first minute of post-conditioning decay compared to the preconditioning baseline. LTP was calculated as the % change of fEPSP slopes of the last five minutes of post-conditioning decay normalized the pre-conditioning baseline.

3. RESULTS

All data are represented as the mean ± SEM. Where n = A;B, A equals the animal number and B represents the slice number. In all other cases, where n = C, C represents the number of animals unless otherwise stated.

3.1 IF rats have relatively heavier brains despite weighing less and having smaller livers

Following the 3-week IF protocol, both FR and control rats gained weight over the 21-day period (Figure 3.1). Nonetheless, excluding day 1 of the fasting protocol, the average bodyweight between groups (n = 16 animals per group) differed significantly from day 2 onwards with the controls weighing significantly more by the end of the protocol ($p < 0.001$). Prior to electrophysiology experiments, both total brain and liver weights were taken within 1 hour prior to the animals' feeding window at the end of their 22-hour fasts. Food restricted animals (n = 4) had significantly heavier total brain weights relative to their body weights when compared to control animals (n = 4) ($p = 0.213$; Figure 3.1B). On the other hand, FR animals (n = 4) were found to have lighter relative liver weights when compared to controls (n = 4) ($p = 0.0268$; Figure 3.1C).

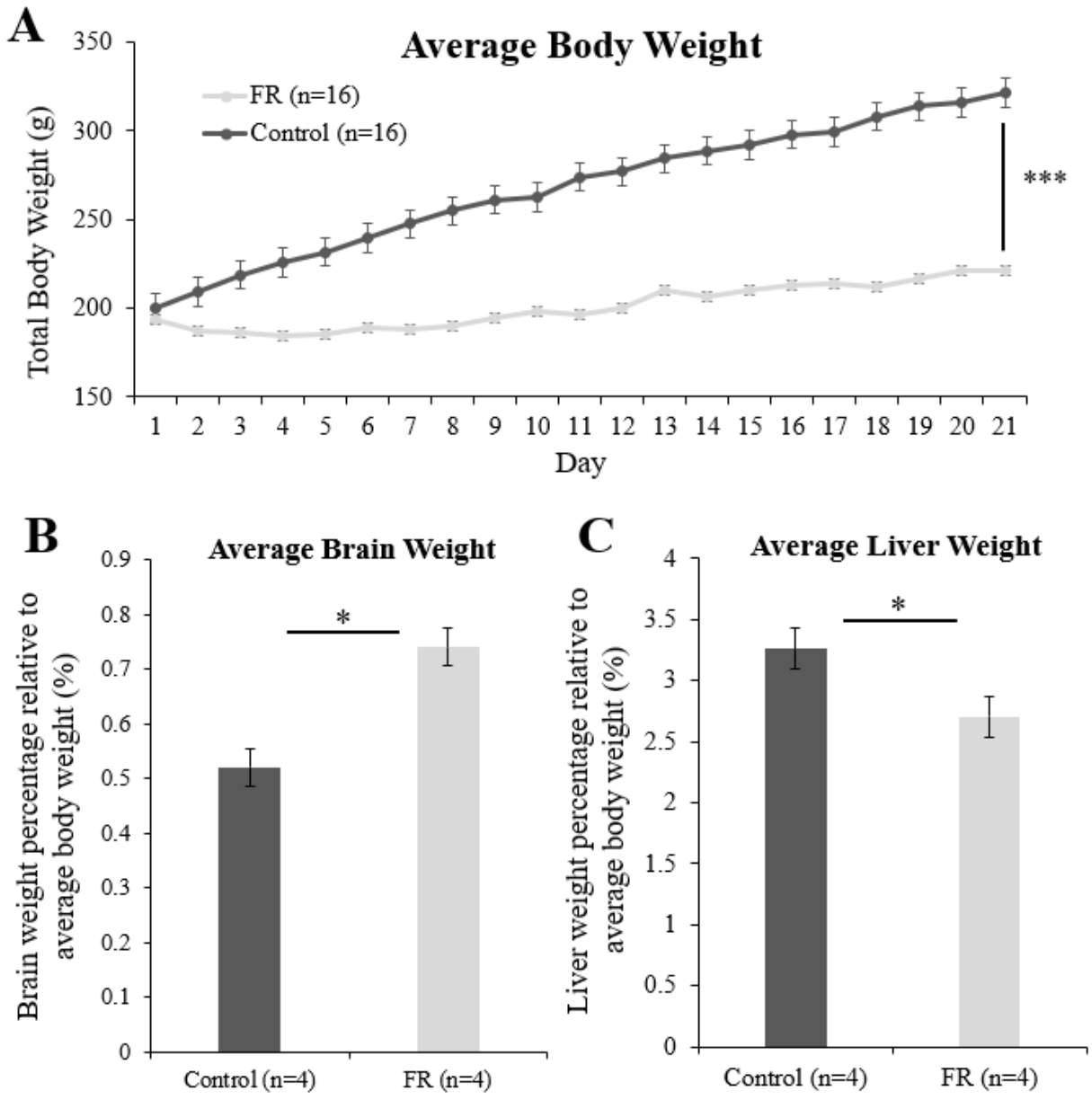

Figure 3.1 - Body/Physical measurements of Adult Male Sprague Dawley Rats following a Three-week Intermittent Fasting Protocol. (**A**) Tracking of Average body weight gain of 16 male Sprague Dawley rats over the three-week IF protocol for both food restricted (FR)and control rats. (**B**) Average Brain weight, including entire cerebral cortex and cerebellum, compared between food restricted and control animals, calculated as a percentage of each animal's brain to body weight ratio. (**C**) Average Liver weight, as the entire liver, compared between food restricted and control animals, calculated as a percentage of each animal's liver to body weight ratio. For all A, B, and C, each n represents a single animal and each error bar represents the standard error of the mean (SEM).

3.1 Input/Output and Paired-Pulse Analysis

In order to determine whether IF affects NT release or postsynaptic responsiveness we examined PPRs and I/O curves, respectively (Figure 3.2). Both PPRs, for PP_{50} and PP_{100}, were significantly augmented in the IF conditions [(1.072 ± 0.0330, n = 4;11) vs (1.117 ± 0.0356, n = 4;9), respectively] in comparison to controls [(0.939 ± 0.0322, n = 5;12) vs (1.007 ± 0.0284, n = 5;12), respectively] ($p = 0.0091$ and $p = 0.0382$, respectively). No significant differences were found at any of the tested pulse widths between IF (n = 4;12) and control animals (n = 4;12) for the I/O data using a two-way repeated measures ANOVA.

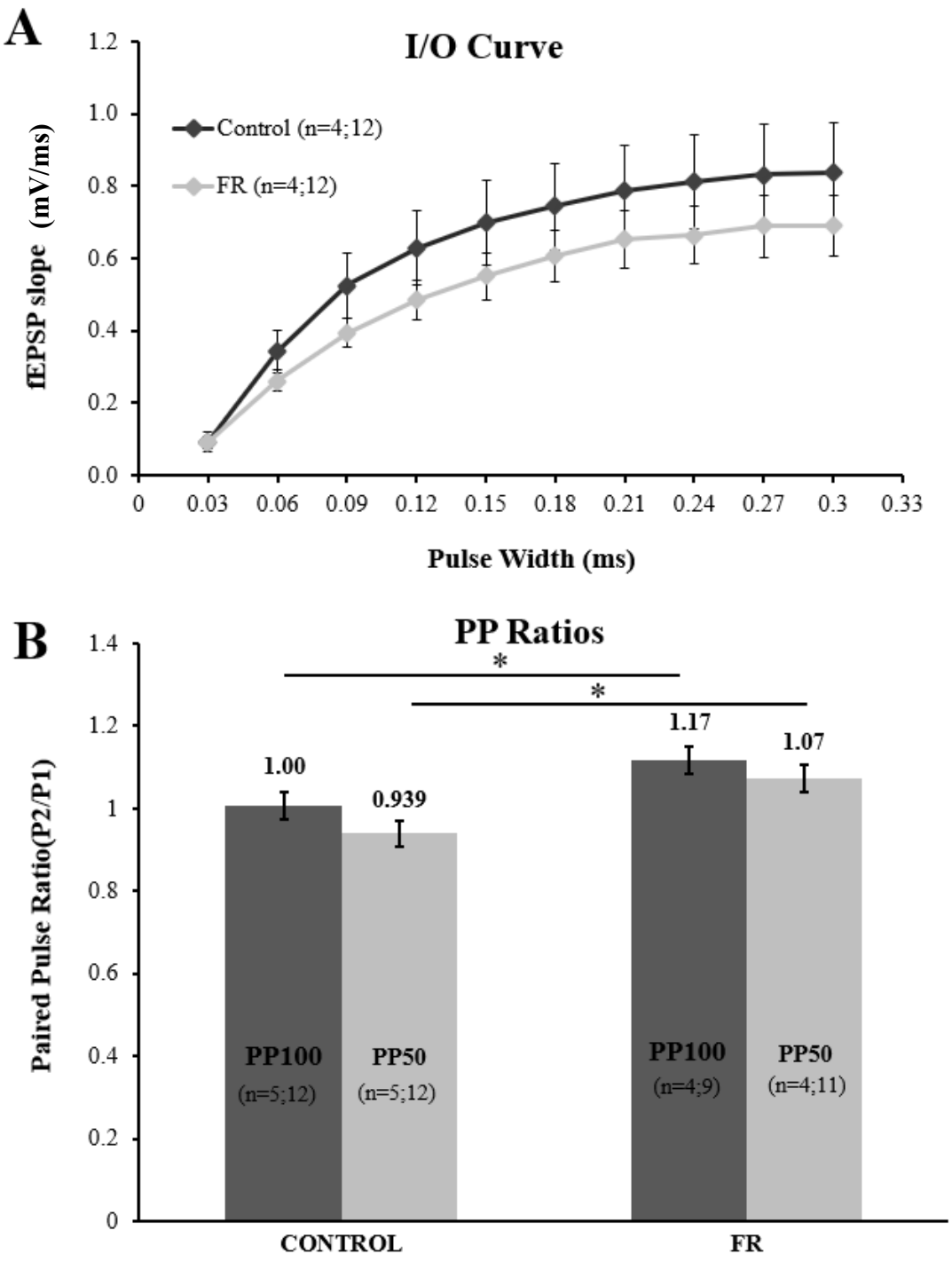

Figure 3.2 - Synaptic Transmission in the Medial Perforant Path Following Intermittent Fasting. Input/output (I/O) function and paired-pulse ratios (PPR) for adult male food restricted (FR) and control rats. Intermittent fasting did not have a significant effect on postsynaptic response but did on NT release, indicated by the paired pulse facilitation (PPF) seen in the FR condition. Data presented as means ± SEM and n = animal number; slice number.

3.3 IF enhances LTP

Measures of short and long-term potentiation were evaluated following the delivery of a CS in the form of HFS (4 trains of 50 stimuli at 100Hz) in the presence of 20 μM Bicuculine.

Following stable pre-conditioning baseline (-20-0 min) and HFS stimulation, indicated by the green arrow at time 0 (Figure 3.3E), PTP, within the first minute of post-conditioning, was noted to have a 74.5 ± 8.01 % change in fEPSP slope relative to pre-conditioning for Control MPP (n = 4;13), 94.5 ± 18.7 % for food restricted (FR) MPP (n = 5;13), with the student's T test revealing no significant difference (p = 0.338; Figure 3.3A). A significant difference between Control MPP (n = 4;13) and FR MPP (n = 5;13) LTP was found, however, at 8.55 ± 9.53 % and 46.4 ± 15.4 %, when fEPSP slopes of the last 5 minutes (55-60) of the postconditioning baseline were compared to the pre-conditioning baseline (p = 0.0306; Figure 3.3B).

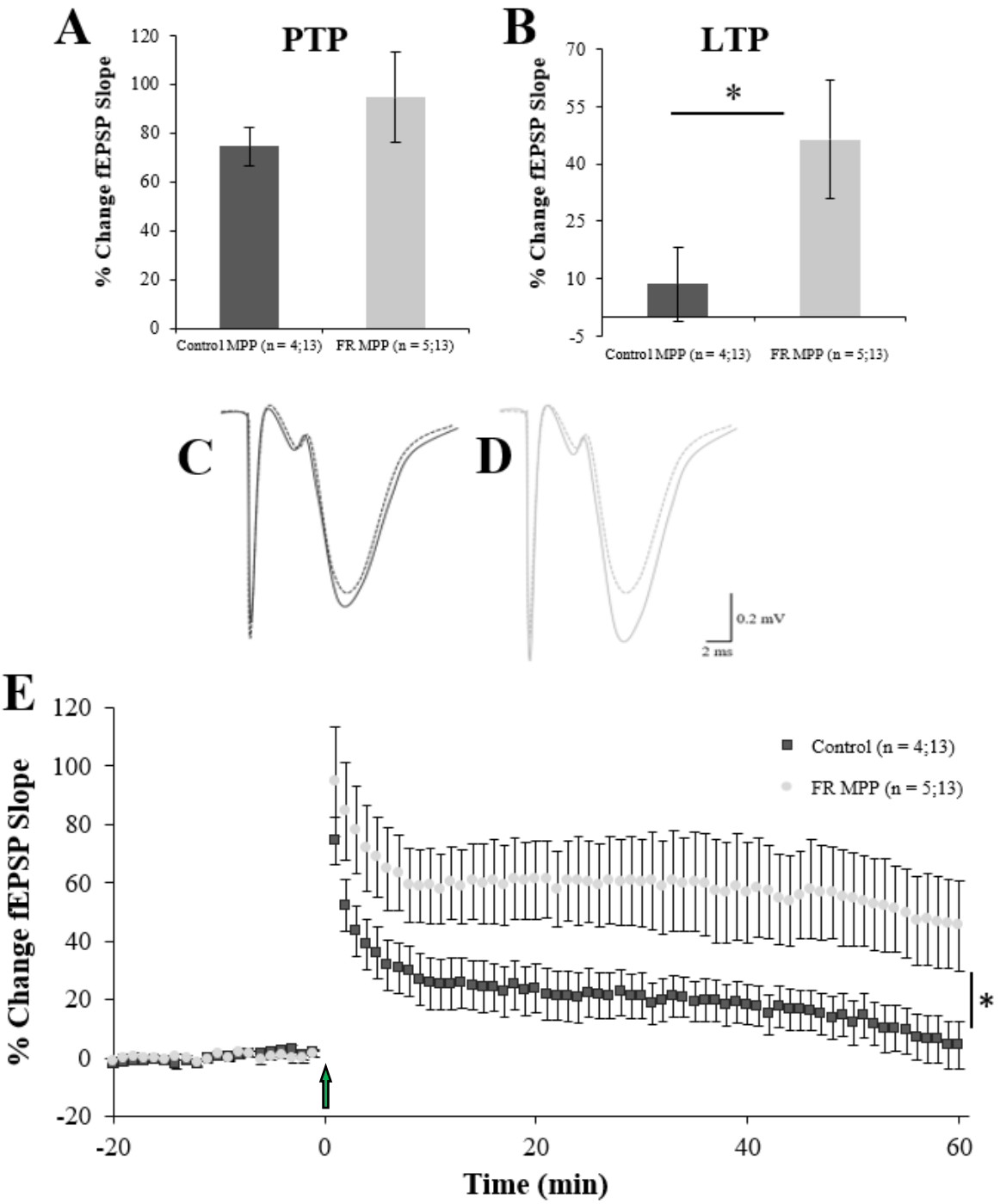

Figure 3.3 – Medial Perforant Path Synaptic Plasticity Results of Post-tetanic Potentiation (PTP) and Long-term Potentiation (LTP) in Intermittently Fasted Rats following High-frequency Stimulation. (**A**) Post-tetanic stimulation (PTP) was measured as the average percentage of change in the fEPSP slope relative to baseline for the first minute of the decay baseline, following the delivery of the high frequency stimulation (HFS) as indicated by the green arrow on (E). (**B**) Long-term potentiation (LTP) was measured as the average percentage of change in the fEPSP slope relative to baseline for minutes 55-60 following delivery of the HFS. Bars represent the average percent change of fEPSP slope and error bars represent the standard error of

the mean, for both (A) and (B). (**C)** Representative trace fEPSP sample in the control for the baseline recording (dotted line) and average LTP (solid line). (**D**) Representative trace fEPSP sample in the FR group for the baseline recording (dotted line) and average LTP (solid line). Scale bars for (C & D) represent 2ms and 0.2 mV for each trace. (**E**) Overall average LTP recordings from the beginning of the pre-conditioning baseline to the end of the post-conditioning baseline. Dots represent the average percent change of fEPSP slope for each minute of recording. Error bars represent the standard error of the mean. Each n = number of animals; number of slices.

4. DISCUSSION

4.1 Overall findings

The overall objective of this pilot study was to test if an IF protocol could enhance synaptic plasticity in the MPP of the DG. Our primary findings show that IF enhances LTP in the MPP (Figure 3.3B & E). We also found that IF induces PPF in the MPP (Figure 3.2B), slows weight gain, increases relative brain weight, and decreases relative liver weight. Altogether, the data we have presented here is purely correlative, but can definitively show that fasting does, in some way, modulate aspects of physiological and brain function

4.2 IF enhances LTP

The primary research point for this study was to test *if* IF can modulate synaptic plasticity in the MPP of the DG in the adult male rat hippocampus. We tested hippocampal brain slices using *in-vitro* electrophysiological recordings using an HFS protocol (4 trains, 50 stimuli, 100Hz) and the $GABA_AR$ antagonist, bicuculine, to unmask any potentiation (Petersen et al., 2013). The use of bicuculine was necessary as research has shown that as granule cells age, they become more strongly influenced by GABAergic activity and otherwise will not be able to elicit LTP such as in younger cells (Lopez-Rojas, Heine, & Kreutz, 2016). Our results show that IF does enhance LTP in the MPP of male adult rats that have undergone a TRF protocol of 2 hours *ad libitum* food access

per day, for 3 weeks. It is important to note that the electrophysiological experiments were conducted within a 1-hour period around when the FR animals would receive their food, which is around the time that the IMS becomes activated (Anton et al., 2018; Mattson et al., 2018). Behavioural anticipation of food occurs around the time of the K-to-G switch of the IMS and is associated with functions of increased protein synthesis, mitochondrial biogenesis, synaptogenesis, and neurogenesis, all of which function to enhance hippocampal LTP through the strengthening of connections between neurons in the trisynaptic circuit (Anton et al., 2018; Mattson et al., 2018; Talani et al., 2015). Similar findings in studies using the same TRF protocol have found enhanced LTP of CA1 pyramidal neurons in addition to increased CA1 mushroom spine densities (Talani et al., 2015) and DG granule cell dendritic spine densities (Lee, Seroogy, & Mattson, 2002; Stranahan et al., 2009), potentially linked to the upregulated BDNF in hippocampal neurons. Talani et al. (2015) found that with enhanced LTP and increased dendritic spine density, BDNF expression in the hippocampus was also upregulated for these FR animals. The upregulation of BDNF may be a contributing factor in the increased presynaptic activation and enhanced glutamate release of FR hippocampal neurons (Falkenberg, Lindefors, Camilli, Metsis, & Ungerstedt, 1996; Marosi & Mattson, 2014). Additionally, though BDNF-LTP, or BDNF induced L-LTP is thought to occur at later stages of LTP and is a protein synthesis dependent form of LTP, well after 60 minutes of HFS induced post-conditioning baseline like we had collected, some research has found that E-LTP, a protein kinase dependent LTP, may require presynaptic BDNF secretion for proper function (Lu et al., 2008).

The IMS induced by fasting has also been shown to prevent age-related deficits in LTP (Eckles-Smith, Clayton, Bickford, & Browning, 2000; Hori, Hirotsu, Davis, & Carpenter, 1992). This result may help explain why we see a loss of potentiation in the adult control animals and

sustained LTP in the adult fasted animals. One explanation is that sustained LTP, a function of upregulated BDNF, is thought to be associated with adult neurogenesis of granule cells the DG which strengthens and increases the overall connections of projections arising from the EC to dendritic spines of DG granule cells (Estrada, 2009). Therefore, if BDNF is upregulated via BHB as a result of the G-to-K switch from IF, then we should see a higher percentage of sustained LTP in the FR group, even at stages of E-LTP, which our results do show.

Though this paper did use behavioural assays, other researchers have found that FR rats showed enhanced performance on long-term spatially oriented memory tasks, in conjunction with showing increased hippocampal LTP and hippocampal dendritic spine densities (Ingram, Weindruch, Spangler, Freeman, & Walford, 1987; Rich et al., 2010). This result aligns with our finding of enhanced MPP LTP of FR rats, in that the MPP is thought to play a role in the consolidation of spatial memories within the hippocampus (Hunsaker et al., 2007).

4.3 Short Term Plasticity; IF induces PPF but not PTP in the MPP

Interestingly, no significant difference in PTP between FR and control rats following the HFS protocol were found. Intermittently fasted animals, did, however, show significant PPF when compared to the controls. While both PPF and PTP increase the probability of quantal neurotransmitter release from the presynaptic membrane, the underlying mechanisms are different (Andersen et al., 2009; Purves et al., 2012). Post tetanic potentiation is mediated by presynaptic activation of protein kinases and the buildup of calcium presynaptic axon terminal while PPF is caused by the presence of residual Ca^{2+} (Purves et al., 2012). Fontán-Lozano et al. (2007) found that fasting induced PPF in the CA regions and that PPRs of different inter-intervals did not differ significantly for FR groups indicating that neuronal excitability to a second pulse is augmented in hippocampal neurons of subjects who underwent an IF protocol. Other research by Thio et al.

(2010) and Koranda, Ruskin, Masino, & Blaise, (2011) looking at the effects of ketone metabolism on paired pulse plasticity in the perforant path of the DG, however, found no significant PPF. Overall, our data contradicts some the findings of the current research in the literature in that we do see facilitation occurring in the MPP of the DG. Nonetheless, our data are completely correlative and we do not have enough definitive evidence to say if the PPF that we see in the FR condition is the result of extra residual calcium being left over in the presynaptic terminal as the result of IF and also that PPF occurs in the DG region of the hippocampus in addition to the CA region as a result of IF.

4.4 IF slows weight gain, increases relative brain weight, and decreases relative liver weight

Our results show that over the 3-week intermittent fasting period whereby male rats were subject to fasting 22 hours a day with a 2-hour eating window where they were given *ad libitum* access to food and water, they weighed significantly less, on average, than the controls throughout the entire fasting protocol except on the first day of the protocol. Interestingly, the FR animals gained weight, overall, but at a much slower rate, on average, than when compared to control animals which were given *ad libitum* access to food and water 24 hours per day. We originally hypothesized that there would be an initial drop in body weight in the FR condition due to experimental conditioning and we correspondingly saw this from our data. With regards to overall bodyweight gain or loss, we hypothesized that FR rats would weigh less than controls overall. This hypothesis was also supported by our data and a number of other studies looking at IF protocols on rodents. Most research has found that with most IF styles of eating in rats and mice, whether it be ADF or TRF, the fasted group always weighed significantly less than the control group and either maintained body weight or slightly gained weight, with higher lean body mass to fat ratios (Anson et al., 2003; Fontán-Lozano et al., 2007; Halagappa et al., 2007; P Mattson & Wan, 2005;

Wan, Camandola, & Mattson, 2003, 2018). It has been proposed that the difference in body weights between seen between controls and FR animals is a result of how IF protocols can lead to an increased average lifespan of up to 40% in male rodents, seen through improved cardiovascular system function and through the processes that come as a result of intermittent metabolic switching such as improved glucose and insulin sensitivity, increased overall systemic BDNF levels, increased mitochondrial biogenesis, and increased cellular autophagy and DNA repair (Chung et al., 2016; K & M.p, 2014; Mager et al., 2006; Mattson et al., 2017). These functions, in conjunction with a more subtle body weight gain, seen in the FR animals, compared to the faster body weight gain in control animals, is proposed to help protect against major chronic diseases such as obesity, diabetes, cancer, and Alzheimer's (Goodrick, Ingram, Reynolds, Freeman, & Cider, 1983; Mattson et al., 2018). Although we did not do biochemical assays on both the brain and livers extracted from our animals, we did measure the relative weight of these organs, to each respective animals' bodyweight, and found that FR animals had significantly lighter livers and heavier brains. The increased brain weight is nothing more than a correlative finding, but the decreased liver weight does align with research from the literature, suggesting that the decrease in liver weight may result from glycogen store depletion as a result of IMS induced via fasting (Anton et al., 2018).

4.5 Limitations and Future Directions

Overall, this study was conducted as a pilot study looking at how and if, IF influences MPP synaptic plasticity. Our original research objectives were to test if IF causes any sort of modulation to MPP LTP in the DG, prior to looking at any sort of mechanistic influence. Since our findings do show that IF does have an influence on both morphological development and hippocampal plasticity of adult male rats, further research should be conducted to determine the mechanisms for what kind of plasticity is being induced by IF, and what sort of biochemical processes are at

play. In other regions of the hippocampus such as the CA1, research shows that IF induces strong CB_1-dependent LTP in adult male rats (Dazzi et al., 2014), however LTP in the MPP of the DG has not been shown to be cannabinoid dependent (Wang et al., 2016). This leads us to the speculation that the sustained MPP LTP seen in our FR may be induced via other mechanisms, such as BDNF-LTP, as BDNF plays a major role in DG granule cell dendritic proliferation, increased spine density, and upregulation of glutamate release at glutamatergic synapses; BDNF is upregulated by IMS induced via IF protocols (Anton et al., 2018; Bramham, 2007; Duan et al., 2003; Farmer et al., 2004; Mattson et al., 2017, 2004; Peters, Reisch, & Langemann, 2018; Talani et al., 2015). We still have yet to test blood and liver samples for glycogen, ketone, and BDNF levels but this is the next step to our research. Biochemical confirmation through these tests will further support our findings, as will prolonging electrophysiological recordings from 60 minutes to 240 minutes to test for properties of L-LTP, indicative of sustained BDNF activity proposed by prior IF research. Lastly, though we anecdotally noticed that the FR animals were more physically active and alert, behavioural experiments, including the Barnes maze test should be conducted as an objective way to test long-term spatial memory of FR animals, as enhanced MPP LTP is considered to be a potential contributing factor in enhancing spatial memory (Hunsaker et al., 2007).

5. CONCLUSIONS

This thesis provides evidence that IF induces both morphological and synaptic plasticity changes. The major findings of this paper show that IF results in a decrease in the rate of body weight gained, an increase of relative brain weight, a decrease of relative liver weight, induces significant PPF in the MPP, and enhances and sustains LTP in the MPP. As a pilot study, confirmation of IF induced synaptic plasticity, along with the morphological changes seen, is enough evidence to support future research to investigate the mechanisms behind how and why IF induces the changes that we see.

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