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Autophagy Mediates the Effects of Physical Exercise on Learning and Memory through Activation of Hippocampal BDNF

By

Vanessa Jabr

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Student Name: Vanessa Jabr I.D. #: 201407879

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The undersigned certify that they have examined the final electronic copy of this thesis and approved it in Partial Fulfillment of the requirements for the degree of:

Master of Science in the major of Biological Sciences

Thesis Advisor's Name: Dr Sama Sleiman

Signature: [redacted] Date: 30 / 07 / 2020
Day Month Year

Committee Member's Name: Dr Joseph Stephan

Signature: [redacted] Date: 30 / 07 / 2020
Day Month Year

Committee Member's Name: Dr Sima Tokajian

Signature: [redacted] Date: 30 / 7 / 2020
Day Month Year

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Autophagy Mediates the Effects of Physical Exercise on Learning and Memory through Activation of Hippocampal BDNF

Vanessa Jabr

ABSTRACT

Physical exercise is known to enhance learning and memory formation in the brain. These positive outcomes are mediated through the induction of the expression of a growth factor, brain derived neurotrophic factor (BDNF), in the hippocampus. BDNF promotes cognitive behaviors and induces brain plasticity. Previous work has also reported that stimulating autophagy can restore cognitive ability. In this study, we demonstrated that a short-term voluntary exercise paradigm is sufficient to upregulate autophagy in different brain regions in an age-dependent manner. This increase in autophagy was correlated with enhanced spatial learning and memory formation particularly in mature adult mice (10-week-old). Indeed, we showed that short-term voluntary wheel running increases the protein expression levels of the autophagy marker, protein light chain 3 (LC3B), in the hippocampus of 10-week-old mice. We used the Morris Water Maze to evaluate spatial learning and memory performance in mice belonging to different age groups. Our work revealed that 10-week-old exercising mice that were treated with a brain-permeable autophagy inhibitor during the behavioral test showed cognitive deficits in the maze suggesting that induction of autophagy is necessary for exercise-induced learning and memory formation. Interestingly, we also found that inhibition of autophagy in exercising 10-week-old mice decreases BDNF protein levels in the hippocampus as compared to the control exercising group. Overall, our results suggest that BDNF acts downstream of exercise-induced autophagy to promote learning and memory formation in mature adult mice. We found that this pathway is not conserved in juvenile or middle-aged mice.

Keywords: Voluntary exercise, autophagy, BDNF, learning, memory, aging.

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Literature Review

1.1 Exercise

1.1.1 The positive effects of exercise on the brain

It is well known that exercise has many beneficial effects on our physical as well as mental health. As a response to physical exercise, structural and functional changes occur in the brain affecting gene expression, and hence, positively impacting cognitive function. Exercise enhances long-term potentiation (LTP) and promotes synaptic plasticity and neurogenesis (van Praag, Christie, Sejnowski and Gage, 1999 ; Bettio, Thacker, Hutton and Christie, 2019 ; Farmer et al., 2004 ; Vaynman, Ying, Yin and Gomez-Pinilla, 2006). Numerous studies have demonstrated that physical exercise enhances learning and memory formation and have described its role in decreasing cognitive deficits through the activation of hippocampal brain-derived neurotrophic factor (BDNF) signaling pathway (Berchtold, Castello and Cotman, 2010 ; Vaynman, Ying and Gomez-Pinilla, 2004 ; Ding et al., 2006 ; Sleiman, 2016). In fact, physical exercise delays the onset, alleviates the symptoms and limits the progression of neurodegenerative diseases such as Alzheimer's disease (Radak et al., 2010 ; Adlard, 2005 ; Law et al., 2018) and Parkinson's disease (Tajiri et al., 2010 ; Frazzitta et al., 2013). Moreover, exercise promotes resilience to chronic stress and decrease anxiety-like behaviors; therefore protecting against depression (Duman, Schlesinger, Russell and Duman, 2008 ; Mul et al., 2018 ; Zhang et al., 2019 ; Patki et al., 2014).

1.1.2 Exercise mechanism of action in the brain

In response to exercise, the expression of several growth factors is upregulated in the brain, especially in the hippocampus (Chieffi et al., 2017). Examples of these factors include the vascular endothelial growth factor (VEGF) and the insulin-like growth factor (IGF-1) that are involved in neurogenesis (Fabel et al., 2003 ; Trejo, Carro and Torres-Alemán, 2001), and most importantly neurotrophins, such as BDNF, that have significant effects on brain plasticity and cognitive function (Vaynman, Ying and Gomez-Pinilla, 2004; Brigadski and Leßmann, 2014).

1.2 Neurotrophins

1.2.1 Overview

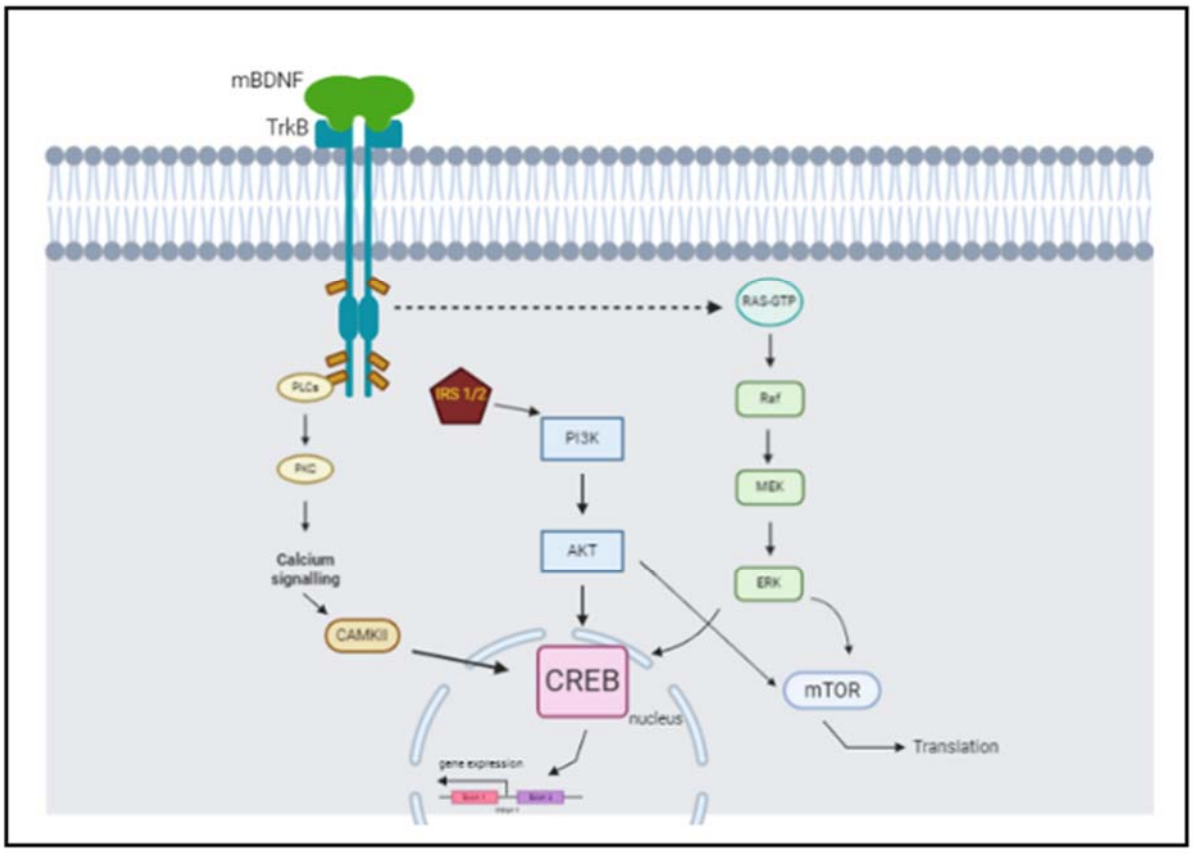
Neurotrophins are a family of growth factors that activate signaling pathways in the nervous system. The nerve growth factor (NGF), BDNF and neurotrophins 3 and 4 (NT-3, NT-4), belong to this family (Hallböök, 1999). Neurotrophins play an important role in neuronal survival during development and continue to modulate neuronal growth and survival, synaptic plasticity and long term potentiation (LTP) in adulthood (Huang and Reichardt, 2001). Neurotrophins bind to 2 different types of receptors, the tropomycin receptor kinase (Trk) family and the p75 neurotrophin receptor (Hallböök, 1999). NGF specifically binds to TrkA, while BDNF and NT-4 bind to TrkB, and NT-3 to TrkC (Lewin and Barde, 1996). BDNF is highly expressed in the central nervous system (CNS), notably the hippocampus and cortex of the brain, and has become the main focus to researchers when attempting to treat neurodegenerative diseases since it is involved in neurogenesis, plasticity and memory (Huang and Reichardt, 2001; Kowiański et al., 2017). BDNF expression is activity-dependent revealing its importance in the adult brain (Mitre, Mariga and Chao, 2017). When the *bdnf* gene is transcribed, the precursor protein proBDNF is produced. This is later converted to the mature form mBDNF. The two products, proBDNF, and mBDNF bind different receptors (Piepmeier and Etnier, 2015), inducing distinct signaling pathways, that have opposite roles in the nervous system. ProBDNF binds the p75 receptor inducing signaling mechanisms involved in long term depression (LTD), apoptosis and retraction of the dendrites, while mBDNF binds to TrkB and induces signaling mechanisms that enhance LTP, cell proliferation and dendritic growth (Erickson, Miller and Roecklein, 2012 ; Phillips, 2017).

1.2.2 Structure and function of BDNF

In rodents, the *bdnf* gene is composed of 9 exons. Each one of the first 8 exons is alternatively spliced to a common coding exon IX leading to a variety of transcripts that encode the same protein, BDNF. Regulation of *bdnf* expression is complex since it is tissue-specific and stimulus-dependent. This is due to the presence of several distinct promoters and the variety of transcription factors that regulate its expression (Aid et al., 2007). In fact, promoter I was shown to be induced in the hippocampus after physical activity (Tabuchi et al., 2002; Sleiman et al., 2016) as well as following a fear conditioning paradigm, suggesting that this specific promoter might play an important role in learning and memory formation (Lubin, Roth and Sweatt, 2008).

1.2.3 BDNF Signaling

The binding of mBDNF to the TrkB receptor induces the dimerization and autophosphorylation of its 2 subunits. When TrkB is phosphorylated (pTrk), it is active. The activation of TrkB will subsequently lead to the activation of three main signaling pathways: the mitogen-activated protein kinase (MAPK) pathway, the phosphatidyl-inositol 3-kinase (PI3K) pathway, and the phospholipase C γ (PLCγ) pathway (Segal, 2003). When PLCγ is active, it cleaves lipids to produce diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 triggers the release of high amounts of Ca^{2+} in the cytoplasm. Increased levels of Ca^{2+} activate the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and induce the transcription factor cAMP response element binding protein (CREB). The activation of the PI3K pathway ultimately leads to the induction of CREB through Akt. Finally, the MAPK signal transduction pathway also leads to CREB's activation (Figure 1). As a result, all three pathways converge on the activation of CREB that regulates signaling pathways that induce brain plasticity, resilience to depression, and improved cognitive performance (Segal, 2003).



<u>Figure 1:</u> Three different signaling pathways are activated by BDNF and they all converge on CREB activation.

This figure includes the following abbreviations: CREB: cAMP-calcium response element binding protein, PLCɣ: phospholipase C, IRS1/2: insulin receptor substrates 1/2, PI3K: phosphatidylinositol 3-kinase, MEK: MAP/Erk kinase, ERK: extracellular signal-regulated kinase, mTOR: mammalian target of rapamycin.

1.2.4 Role of BDNF in promoting learning and memory

In the adult brain, BDNF regulates neurogenesis and synaptic plasticity such as LTP (Autry and Monteggia, 2012). When BDNF signaling is genetically or pharmacologically inhibited, learning and memory formation are impaired (Yamada, Mizuno and Nabeshima, 2002; Gomez-Pinilla, Vaynman and Ying, 2010). Along with *bdnf*, the expression of its receptor *trkB* is also affected. When a spatial memory is acquired, TrkB is activated by phosphorylation and this effect is lost upon inhibition of learning (Yamada, Mizuno and Nabeshima, 2002). Another study reported that rodents trained in a fear conditioning paradigm exhibited increased hippocampal BDNF levels induced by memory extinction (Rosas-Vidal, Do-Monte, Sotres-Bayon and Quirk, 2014). In addition, rats treated with intra-hippocampal BDNF prior to a behavioral task such as the water maze performed better than control groups (Cirulli, Berry, Chiarotti and Alleva, 2004). Hence, learning and memory are greatly affected by BDNF signaling.

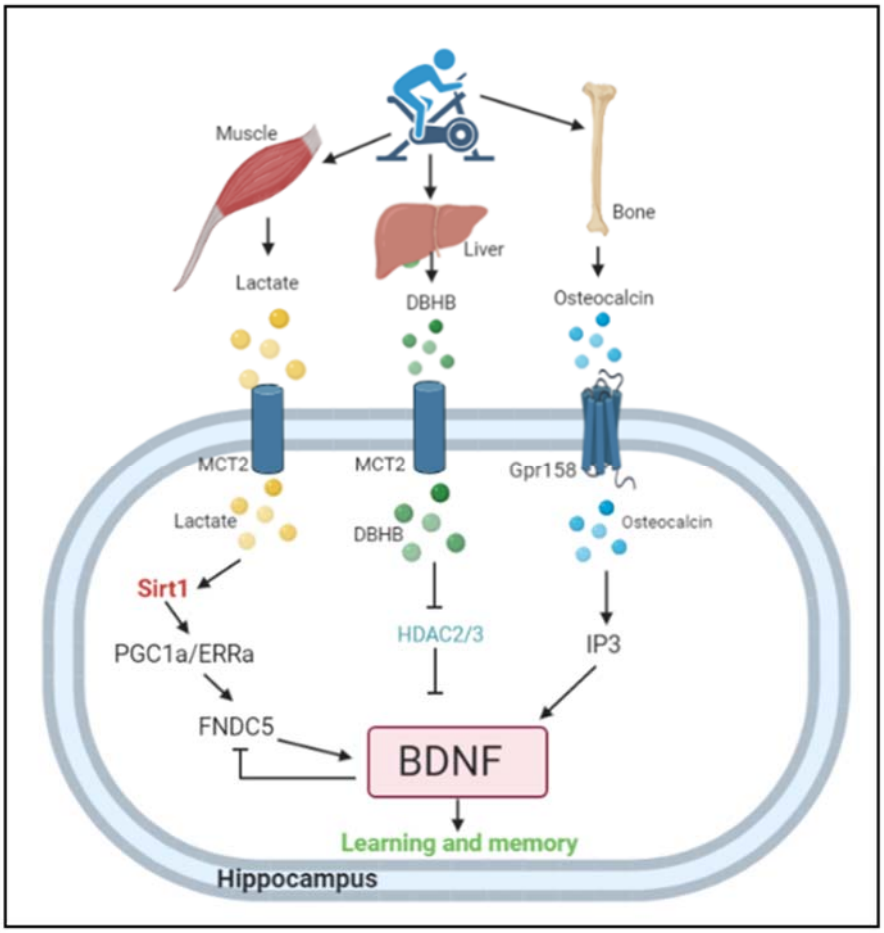
1.2.5 Metabolites that mediate the positive effects of exercise through BDNF induction

Some known mechanisms by which physical exercise induces *bdnf* expression involve the release of endogenous metabolites from different tissues such as the muscle, liver or bones (Figure 2). These molecules mediate the positive effects of exercise on the brain by crossing the blood-brain barrier (BBB) and activating neuronal signaling mechanisms that eventually lead to increased hippocampal BDNF levels. Previous work from our laboratory showed that lactate, which is produced in muscles in response to exercise, crosses the BBB and makes its way to the brain. In the hippocampus, lactate activates the histone deacetylase sirtuin 1 (SIRT1), which induces the expression of *bdnf* through a PGC1a/FNDC5 pathway (El Hayek et al., 2019). Indeed, one mechanism by which *bdnf* expression is induced involves the action of a myokine Fibronectin type III domain-containing protein 5 (FNDC5). The downstream effectors through which FNDC5 induces *bdnf* expression are not identified. Indeed, fndc5 mRNA expression in the hippocampus is induced by a complex between estrogen related receptor alpha

(ERRa) and the transcriptional coactivator (PGC1a) (Wrann et al., 2013). After exercise, lactate increases PGC1a levels in the hippocampus. PGC1a interacts with ERRa forming a complex that binds to the promoter of *fndc5*. FNDC5 in turn activates *bdnf* expression in the hippocampus through intermediate signaling pathways (Wrann et al., 2013; Phillips, Baktir, Srivatsan and Salehi, 2014).

Our group also identified another mechanism of exercise-mediated *bndf* induction that involves the ketone body β-hydroxybutyrate (DBHB). Exercise induces DBHB release by the liver into the blood. DBHB levels increase in the hippocampus where it acts as a class I histone deacetylase inhibitor, blocking the recruitment of HDACs 2 and 3 to *bdnf*'s activity-dependent promoter 1, thus activating it (Sleiman, 2016).

In addition to these, osteocalcin is also a molecule released by the bones after physical activity (Mera et al., 2016). Studies have demonstrated that osteocalcin too crosses the BBB, where it plays a role in improving anxiety-like behaviors and cognitive function (Shan et al., 2019). Once osteocalcin reaches the hippocampus of the brain, it interacts with Gpr158, a G-coupled receptor. As a result, IP3 accumulates in the brain and BDNF levels increase (Khrimian et al., 2017; Obri, Khrimian, Karsenty and Oury, 2018). Other studies also demonstrated that the histone-binding protein RbAp48 regulates the expression of Gpr148 and BDNF, leading to the beneficial outcomes related to memory and cognition (Kosmidis et al., 2018).



<u>Figure 2</u>: Metabolites released upon to exercise and the molecular pathways they respectively activate to induce BDNF and promote learning and memory in the hippocampus.

1.3 Autophagy

Autophagy is an intracellular catabolic process involved in the self-degradation of old malfunctioning organelles as well as misfolded proteins and cytoplasmic aggregates. In response to various stresses, autophagy is involved in the recycling and remodeling of cells and their components in order to maintain homeostasis and produce energy (Mizushima and Komatsu, 2011). There are three different types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (Figure 3). In macroautophagy, the cargo is transported to the lysosome through an intermediary membrane that will fuse with the lysosome, while microautophagy is the process whereby the cytosolic components are directly taken up by the lysosome itself through invagination (Mizushima and Komatsu, 2011). CMA is when targeted proteins are translocated along with chaperone proteins as a complex, and taken towards the lysosomal membrane where they are recognized by the receptor LAMP-2A resulting in their unfolding and degradation (Towers and Thorburn, 2016 ; Liang, 2019, Mizushima and Komatsu, 2011). Although macroautophagy is extensively studied in the context of protein homeostasis (proteostasis), aging, and neurodegeneration, it is still unclear how environmental stresses affect autophagy and regulate brain health.

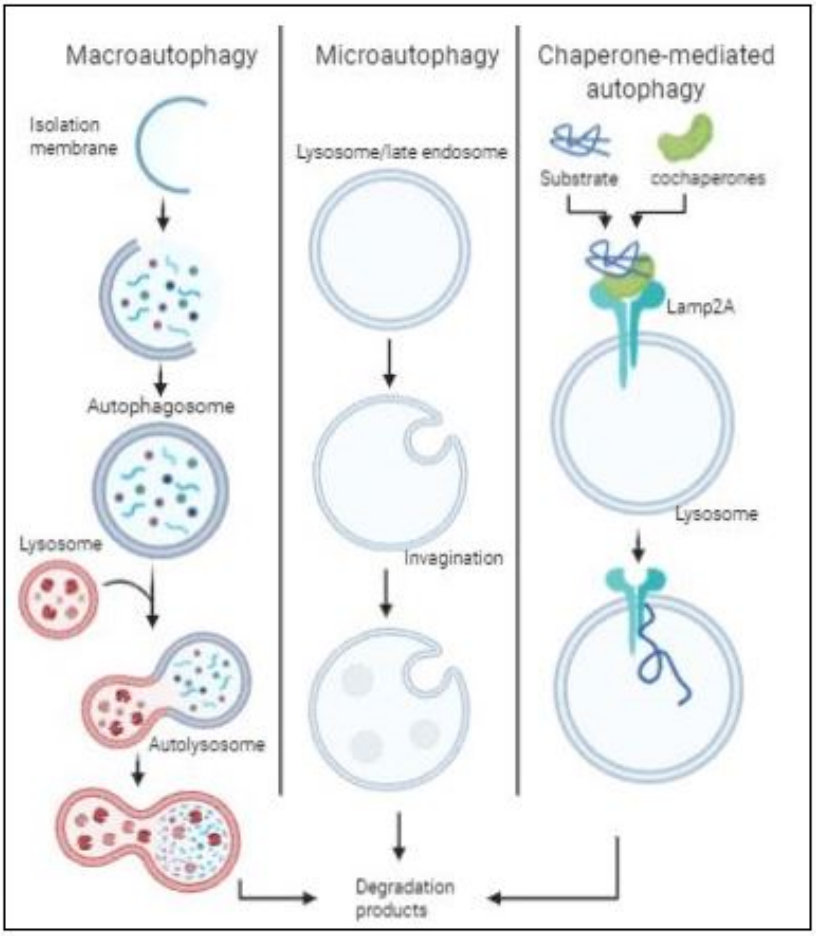


Figure 3: The three different types of autophagy

1.3.1 Autophagy markers

Autophagy requires the formation of a double-membraned vesicle, the autophagosome, which transports the unnecessary, damaged material targeted for degradation towards the lysosome. Their fusion will form an autophagolysosome inside which the engulfed material will be degraded by hydrolases. This process consists of three steps: initiation and elongation, maturation, and fusion. The biomarkers used in the study of autophagy are proteins involved in the first phase, whose final product is the autophagosome. For initiation, a complex containing Vps34, Beclin-1, ambra1 and others is formed, while elongation occurs in two pathways similar to ubiquitin conjugation. One protein whose levels are used as a marker for autophagy is the

microtubule-associated protein light chain 3 (LC3B). It is found in the cytosol of most cells and is cleaved into LC3B-I upon autophagy induction and then converted from the activated LC3B-I form to LC3B-II which is incorporated into the membrane of the autophagosome by the action of autophagy proteins (ATGs). LC3B-II (or LC3B) helps in the fusion of the membranes and the selection of the substances to be degraded; its expression increases during autophagy (Glick, Barth and Macleod, 2010). The autophagy receptor sequestosome 1 (SQSTM1, p62) is another protein that is used as a marker for autophagy, It binds to misfolded proteins that have been previously ubiquitinated and disposes them to decrease toxicity or directs them for degradation (Zatloukal et al., 2002; Moscat and Diaz-Meco, 2009). p62 is also integrated into the autophagosomal membrane and physically links the cargo to the membrane (Glick, Barth, and Macleod, 2010). The levels of both of these proteins have been extensively studied as molecular markers of autophagy.

1.3.2 Autophagy in the brain

Autophagy has a vital role in the brain in terms of neuronal integrity, signaling, and development as well as in regulating synaptic plasticity and memory formation (Liang, 2019; Nikoletopoulou et al., 2017). It is extremely important for autophagy to properly take place in the brain, at least at basal levels, to recycle damaged organelles, and clear harmful and misfolded proteins whose accumulation causes an imbalance in proteostasis and could ultimately lead to neurodegeneration; as a result, constant surveillance of neuronal protein quality is vital to prevent neuronal loss (Eric E Essick, 2010 ; Liang, 2019 ; Kulkarni, Chen and Maday, 2018).

Researchers have linked the development of neurodegenerative diseases to mutated autophagy proteins (Frake, Ricketts, Menzies and Rubinsztein, 2015) and have shown that induction of autophagy in neurons actually rescues the deficits observed and could be targeted for therapy (Barmada et al., 2014). Others have shown the importance of autophagy in regulating synaptic plasticity and cognition, especially memory formation (Liang, 2019; Tripathi, 2019). Rodent studies by Hylin et al. revealed that special

learning causes hippocampal LC3B levels to increase and that the formation of long-term memories requires autophagy (Hylin et al., 2017). Moreover, age-related memory deficits were reversed by stimulating autophagy in the brain (Glatigny et al., 2019; Tripathi, 2019).

1.3.3 Diet and autophagy

Diet, like exercise, has a major impact on brain function. For example, we have recently shown that a high protein diet promotes resilience to chronic social defeat stress (Nasrallah et al., 2019). Interestingly, *in vivo* studies demonstrated that fasting over a short period of time significantly increases levels of autophagosomes formed in neurons, specifically in cortical and Purkinje cells, through upregulating the neuronal signaling pathways of autophagy (Alirezaei et al., 2010). Moreover, food administration in fasting conditions showed neuroprotective roles in rat and mouse models of neurodegenerative diseases and CNS injuries as compared to ad libitum controls (Longo and Mattson, 2014). Recent work established that caloric restriction promotes memory formation by inhibiting autophagy in the hippocampus and that this inhibition was dependent on BDNF signaling (Nikoletopoulou et al., 2017).

1.3.4 Exercise and autophagy

Through autophagy, physical exercise can improve cognition and protect from neurological diseases (Xing et al., 2019). This involves destroying Aβ plaques in APP/PS1 transgenic AD mouse models in response to exercise through enhancing autophagy-lysosomal performance (Zhao et al., 2018). Exercise promotes autophagy not only in the muscle, but also in the brain. This increase in autophagic flux after exercise was demonstrated by increased LC3B levels and reduced amounts of SQSTM/p62 in the cortex (He, Sumpter, Jr. and Levine, 2012). However, the intensity and duration of exercise play crucial roles in activating autophagic pathways (Schwalm et al., 2015). These pathways involve either a boost in autophagic flux upon exercise or induction of

important genes related to autophagy (Halling and Pilegaard, 2017). Interestingly, short-term voluntary exercise induces autophagy in the cortex of 8-12 week-old mice (Rocchi and He, 2017). Other studies have shown that autophagy is impaired in older mice as compared to younger ones, leading to the accumulation of aggregates and damaged organelles in the cells, and consequently, resulting in neurodegeneration (Metaxakis, Ploumi and Tavernarakis, 2018).

1.4 Aging and the brain

Aging is defined by the build-up of damage in the genetic make-up that progressively causes functional decline. Some of its hallmarks include changes in cell-to-cell communication, mitochondrial dysfunction, and deterioration in maintaining proteostasis (López-Otín et al., 2013). Aging is accompanied by a decrease in the neuronal count and synapse formation, as well as a general decrease in plasticity. These alterations lead to cognitive decline in a time-dependent manner. In fact, certain studies showed deterioration in BDNF signaling with increased age and thus poor learning and memory formation (Miranda, Morici, Zanoni and Bekinschtein, 2019). Normally, autophagy eliminates these damaged aggregates to maintain proteostasis in body tissues and the brain; however, many scientists have reported a malfunction in this process relating aging to degeneration (Daniele, Giacomelli and Martini, 2018). For example, in neurodegenerative diseases, the number of autophagy-related genes and proteins decreases and the presence of abnormal autophagosomes increases (He, Lu and Yue, 2013). Based on that, studies reported that repressing autophagy accelerates aging and that inducing autophagy has an opposite effect, extending longevity (Rubinsztein, Mariño and Kroemer, 2011). Physical activity attenuates many of the symptoms of neurodegenerative diseases by increasing BDNF production, even in aged mice. Moreover, exercise is known to have a beneficial impact on autophagy aiming to maintain cellular homeostasis by degrading dysfunctional organelles and protein aggregates. By targeting the known hallmarks of aging, exercise can be a very effective therapeutic approach to delay aging and prevent neurodegeneration (Garatachea et al., 2015).

1.5 Aim of this study

In this study, we hypothesized that voluntary exercise induces autophagy in the brain in an age-dependent manner. We aimed to examine whether the induction of autophagy by exercise promotes spatial learning and memory formation through hippocampal BDNF activation. To assess this, we looked at the induction of autophagy in different brain regions, most notably the cortex and hippocampus of juvenile, mature, and middle-aged mice.

Chapter Two

Materials and Methods

2.1 Experimental Subjects

Male C57BL/6 mice were individually housed in cages with food and water *ad libitum* and maintained on a 12-hour light-dark cycle. Mice of different age groups were used, ranging from 4, 10 and 22-and 32-weeks old. They were divided into sedentary or exercise groups and further classified based on the treatments they received. Animals were sacrificed either directly after a 2-week exercise paradigm or after behavioral tests were performed. Hippocampal and cortical tissues were collected on dry ice and stored at -80°C. This work was approved by the Lebanese American University Animal Care and Use Committee (ACUC).

2.2 Exercise Paradigm

Male mice were divided into either sedentary or exercising groups. Each exercising mouse was housed with a running wheel that they can freely access for a period of 2 weeks. The control mice were also individually housed in cages with the same conditions, but in the absence of a running wheel.

2.3 Treatments

Male mice received intraperitoneal injections of chloroquine diphosphate (50mg/kg), an inhibitor of autophagy that is able to cross the BBB. Chloroquine diphosphate was dissolved in saline and injections were performed only during the behavioral experiments, that is after the completion of the short-term exercise paradigm.

The injections were administered daily,15 minutes prior to behavioral training. Control mice received saline injections.

2.4 Morris Water Maze Test

After 2 weeks of exercise, the special memory of mice was tested using the Morris Water Maze (MWM). This test was performed as previously described (Morris, 1984). Briefly, the maze consists of a pool that is divided into 4 equal quadrants (Q1-Q4). The pool has a platform placed in Q1 as well as visual cues distributed around its circumference. Mice were placed in the pool full of clear water and a visible platform on day 1 to get acquainted with the set up. From day 2 through day 6, white paint was added in order to hide the platform. Learning was assessed over these 5 days based on the escape latency or the period of time in seconds taken by the mouse to reach the hidden platform and escape the water. In this paradigm, the mouse relies on its spatial memory using the visual cues on the borders of the pool to reach the hidden platform and escape the water. Each mouse was given three 1-minute trials, each from a different starting point. The ANY-maze Video Tracking System was used to record the escape latency every day. On day 7, the platform was completely removed from the pool of white paint, and the time spent in the quadrant that previously contained the platform (Q1) was recorded.

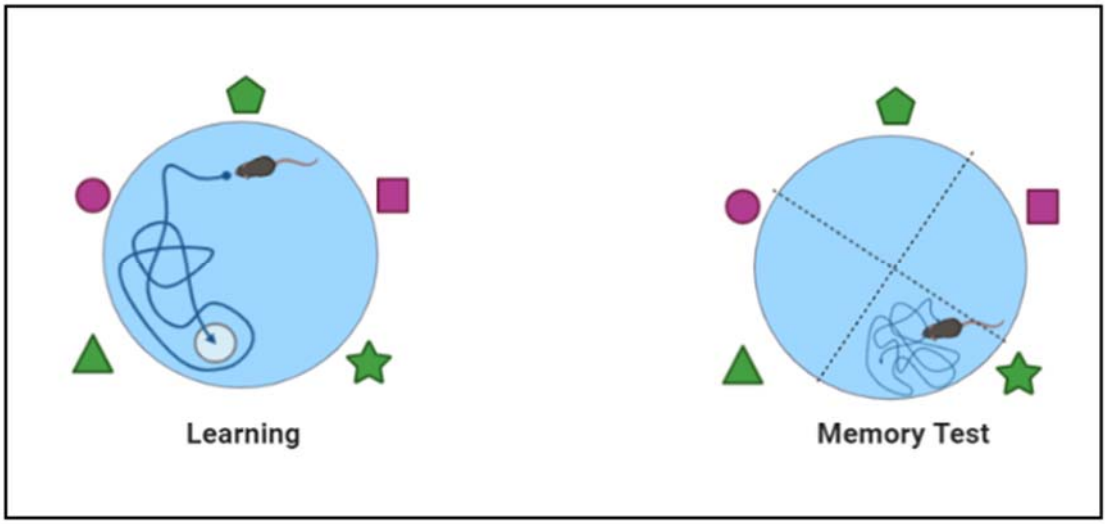


Figure 4: Illustrative schematic of the Morris Water Maze setup.

2.5 Protein Extraction and Western Blot Analysis

Total cellular proteins were extracted from the tissues by preparing a master mix of lysing buffer RIPA-B (1% Triton X-100, 1% SDS, 50mm Tris-Cl, pH 7.4, 500mm NaCl, and 1mm EDTA), protease (Sigma, BioWORLD), phosphatase (Sigma, BioWORLD), and proteasome (MG-132) inhibitors (Sigma). Samples were boiled in Laemelli buffer and were electrophoresed on an acrylamide gel (Bio-Rad) and then transferred to a PVDF membrane using Trans-Blot SD Semi-Dry transfer cell (Bio-Rad). Membranes were blocked using non-fat milk diluted in TBS-Tween. Membranes were incubated either overnight at 4°C or for 2 hours at room temperature. Primary antibodies against LC3B (Abcam), BDNF (N-20, Santa Cruz Biotechnology), and GAPDH (Abcam) will be diluted 1:2000, 1:500, and 1:1000 respectively in blocking buffer. Secondary antibodies (Bio-Rad) were used at a 1:5000 dilution and incubated for 1 hour at room temperature. Three washes of 5 minutes each with TBS-T were performed after primary antibody incubation and again after secondary antibody incubation. Finally, the proteins were detected by chemiluminescence on ChemiDoc (Bio-Rad) using Clarity Western ECL Substrate or Super Signal West Femto. Band quantification and analysis was done with ImageJ software.

2.6 Statistical Analysis

Unpaired T-test and one-way ANOVA were used to assess the statistical significance of the results. A $p<0.05$ was considered to be statistically significant

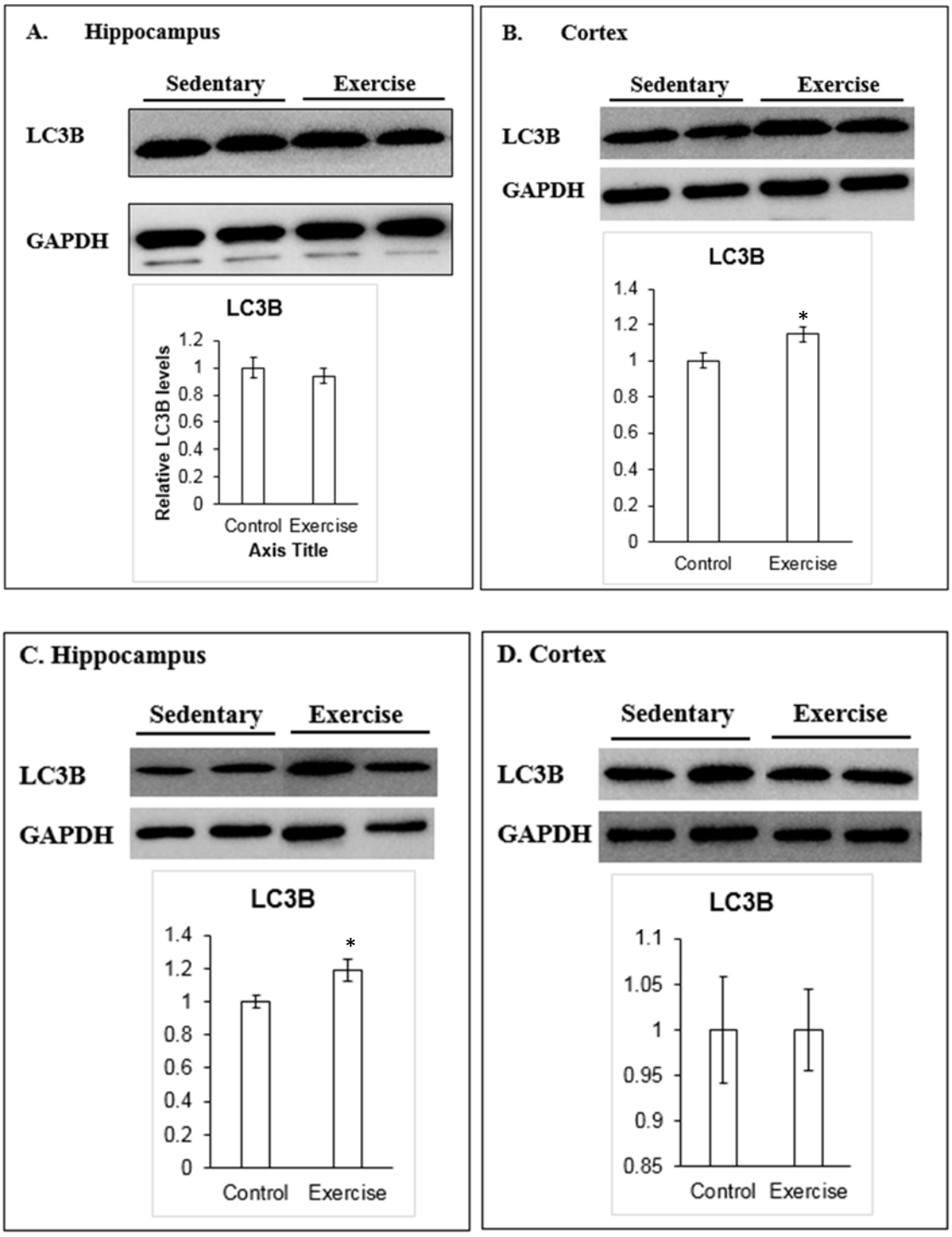
*: $p<0.05$, **: $p<0.01$, ***: $p<0.005$

Chapter Three

Results

3.1 Short-term exercise regulates autophagy protein marker expression in an age-dependent manner

We were first interested in determining how exercise affects autophagy in the brains of mice that belong to different age groups. We studied mice that are juvenile (4-weeks-old), mice that are mature adults (10-weeks-old), as well as middle age mice (older than 22-weeks old) (Jackson et al., 2017). To determine how short-term exercise affects autophagy in the brain across the different age groups, we subjected mice to 14-days of voluntary wheel running (Diederich et al., 2017). At the end of the exercise paradigm, we euthanized the mice and harvested different brain regions, such as the hippocampus and cortex. To assess how exercise affects autophagy in these animals, we compared the protein levels of the autophagy marker LC3B from the hippocampus and cortex of exercising animals to the sedentary animals. We found a significant increase in the protein levels of LC3B in the cortex (Ttest=0.03356), but not the hippocampus (Ttest=0.51644) of 4-week-old exercising mice as compared to control sedentary mice (Figure 5A and B). Our results suggest that exercise activates autophagy in the cortex, but not the hippocampus of juvenile mice. The opposite result was observed in 10-week-old exercising mice. Indeed, we observed a significant increase in the protein levels of LC3B in the hippocampus (Ttest=0.0237), but not the cortex of 10-week-old exercising mice when compared to control sedentary mice (Ttest=0.9987) (Figure 5C and D). Our results suggest that exercise activates autophagy in the hippocampus, but not the cortex of mature adult mice. Interestingly, we did not observe significant effects of exercise in middle age mice (22 weeks old) on LC3B protein levels in neither the hippocampus (Ttest=0.84902) nor the cortex (Ttest=0.71818) (Figure 5E and F).


A. Hippocampus
Sedentary
Exercise
LC3B
GAPDH
LC3B
Relative LC3B levels
1.2
1
0.8
0.6
0.4
0.2
0
Control
Exercise
Axis Title
B. Cortex
Sedentary
Exercise
LC3B
GAPDH
LC3B
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
Exercise
C. Hippocampus
Sedentary
Exercise
LC3B
GAPDH
LC3B
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
Exercise
D. Cortex
Sedentary
Exercise
LC3B
GAPDH
LC3B
1.1
1.05
1
0.95
0.9
0.85
Control
Exercise

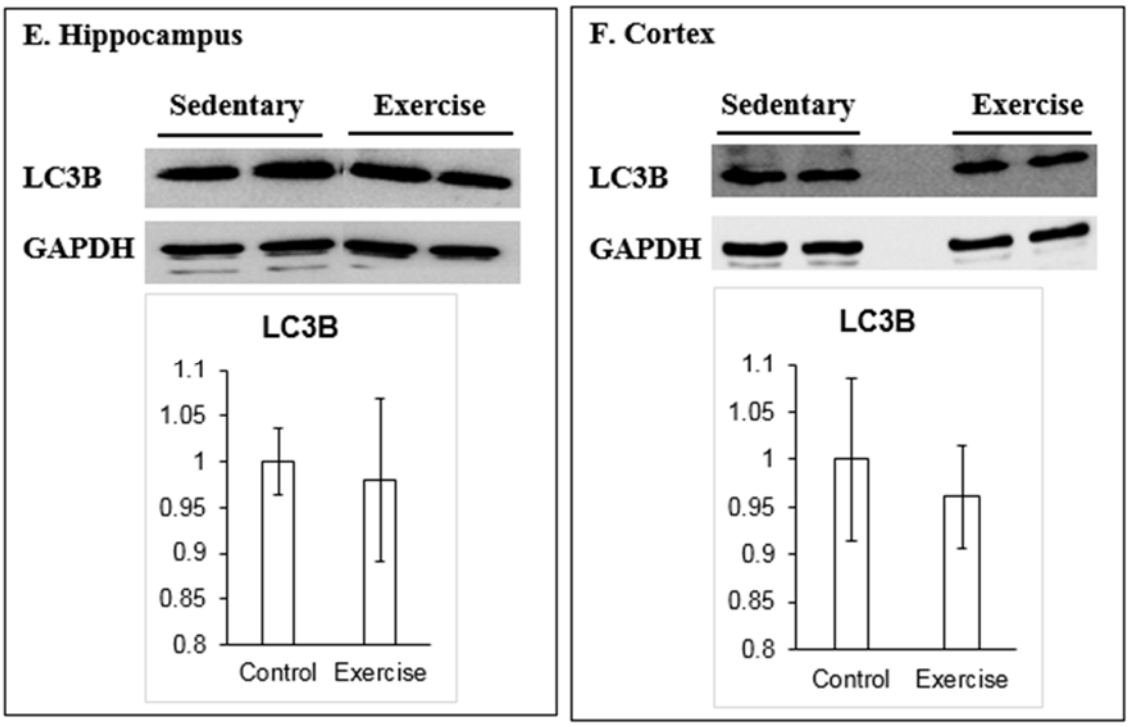
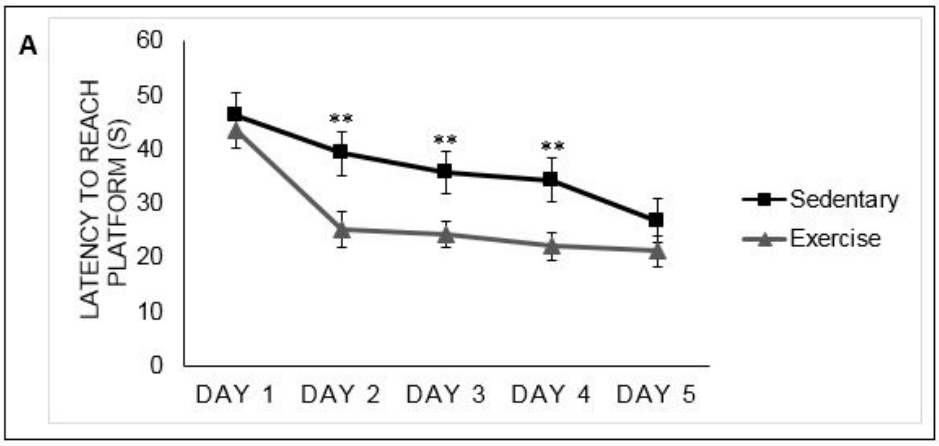


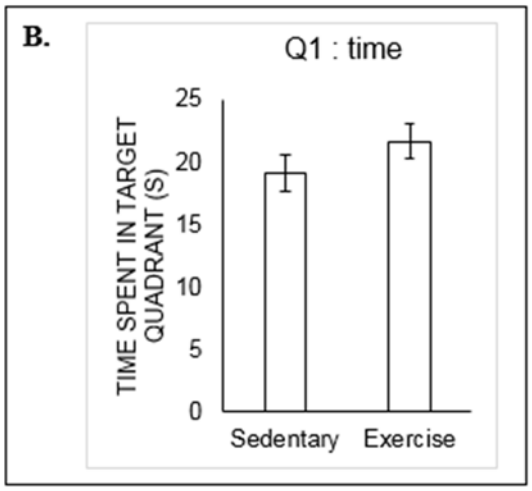
Figure 5: The induction of autophagy upon short-term voluntary exercise differs according to age

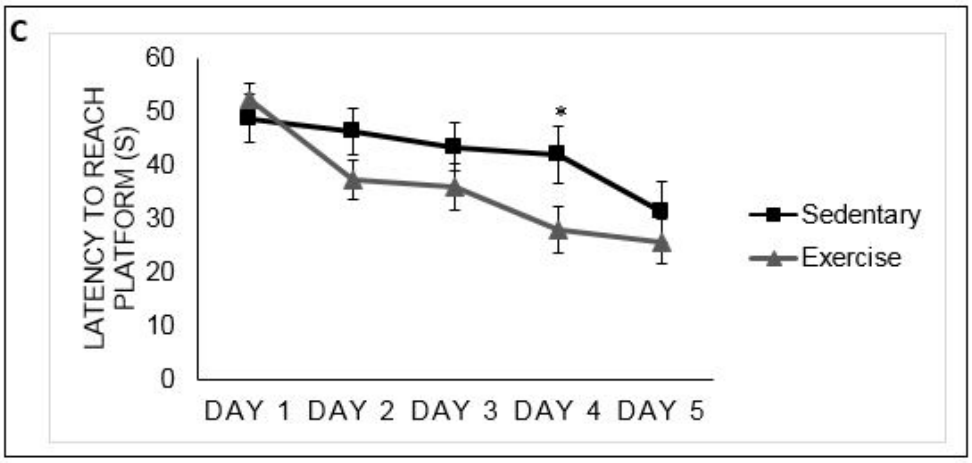
(A) and (B) Representative western blot images and their relative quantification showing the effect of exercise on LC3B levels in the hippocampus and cortex of 4-week-old mice respectively. (B) Statistical significance was measured by unpaired t-test *$p<0.05$. (C) and (D) Representative western blot images and their relative quantification showing the effect of exercise on LC3B levels in the hippocampus and cortex of 10-week-old mice respectively. (C) Statistical significance was measured by unpaired t-test *$p<0.05$. (E) and (F) Representative western blot images and their relative quantification showing the effect of exercise on LC3B levels in the hippocampus and cortex of 22-week-old mice respectively. The first 2 lanes correspond to the sedentary mice while the last 2 lanes correspond to exercising mice. GAPDH was used as a loading control for all.

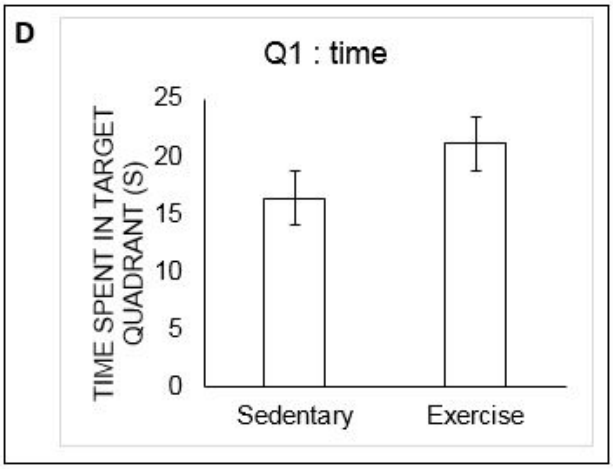
3.2 Short-term exercise improves spatial learning and memory in 4 and 10-week-old mice, but not in 22-week-old mice

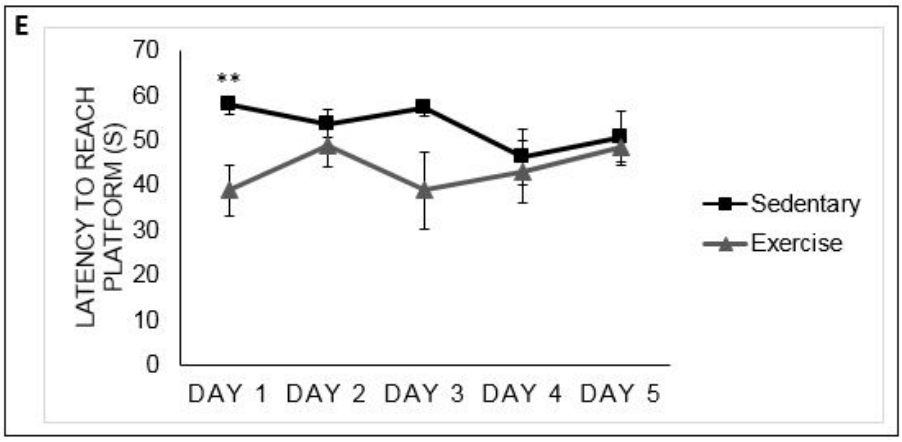
To assess how short-term exercise affects spatial learning and memory formation in mice that belong to different age groups, we used the MWM paradigm. We observed that exercise enhanced learning in juvenile mice as they displayed a shorter escape latency (Figure 6A), however we did not observe any significant enhancement in memory formation in these mice (Figure 6B). In contrast, we observed that exercise enhanced learning in mature adult mice and enhanced memory formation (Figure 6C and D and Figure 7D). In 22-week-old mice, physical activity did not significantly impact the mice's performance in the water maze (Figure 6E and F). Exercising mice outperformed sedentary mice only on the first day of the learning paradigm after which the performance of both groups of mice was equivalent (Figure 6E).

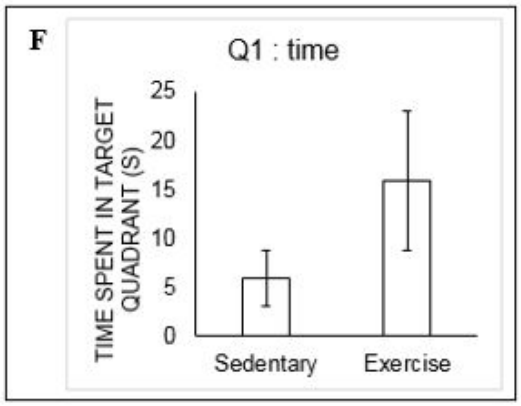



B.
Q1 : time
TIME SPENT IN TARGET QUADRANT (S)
25
20
15
10
5
0
Sedentary
Exercise


C
LATENCY TO REACH PLATFORM (S)
60
50
40
30
20
10
0
*
DAY 1 DAY 2 DAY 3 DAY 4 DAY 5
Sedentary
Exercise


D
Q1 : time
TIME SPENT IN TARGET QUADRANT (S)
25
20
15
10
5
0
Sedentary
Exercise



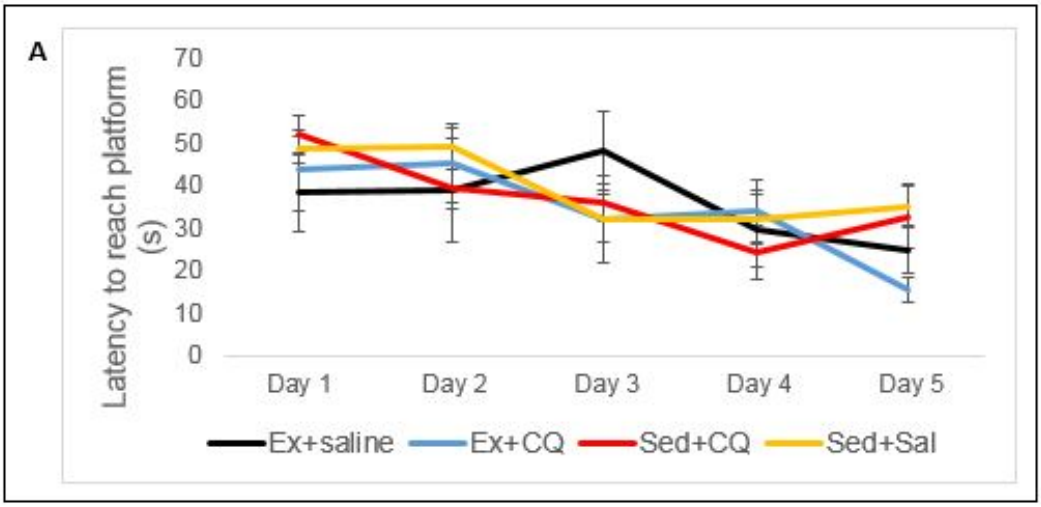


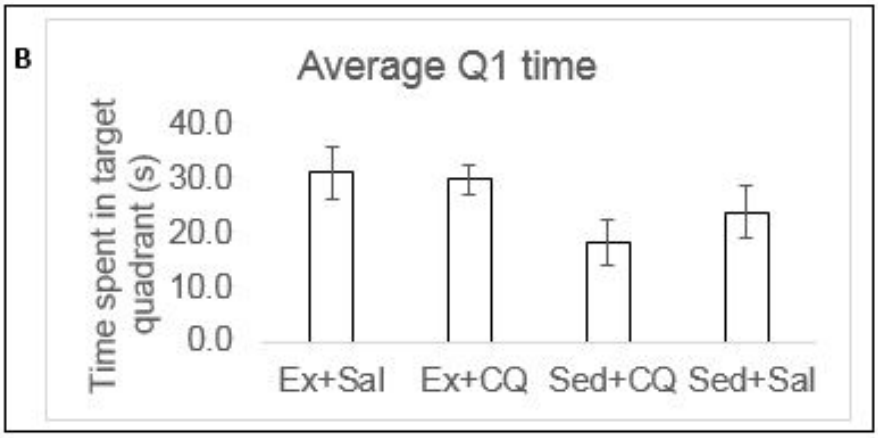
<u>Figure 6</u>: Short-term voluntary exercise enhances special learning and memory formation in the different age groups.

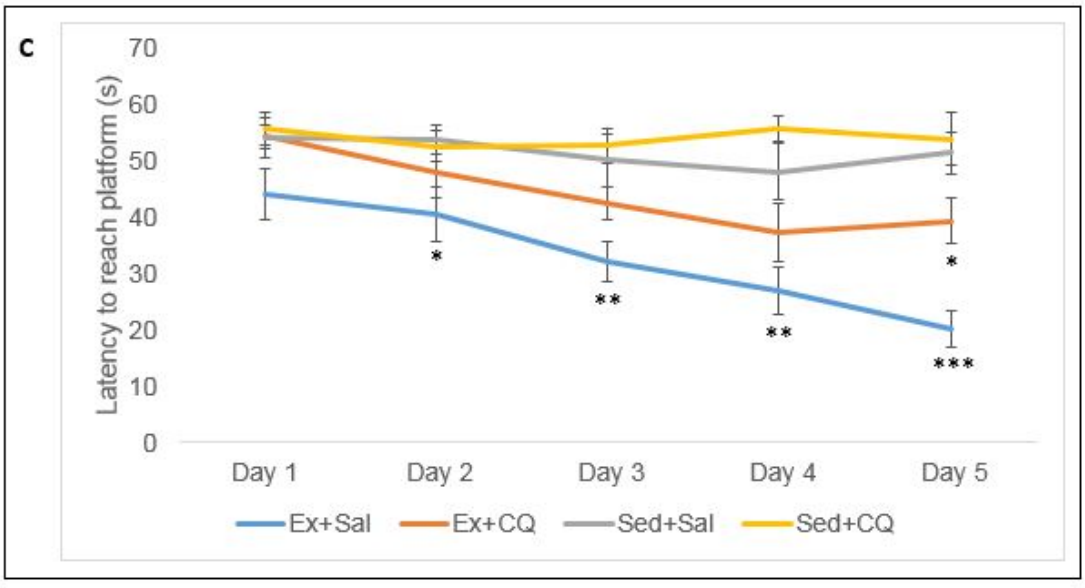
(A) Graph showing the latency period for 4-week-old exercising mice to reach the platform per day as compared to their sedentary group. Statistical significance was measured by unpaired t-test **p<0.01. (B) Graph indicating the average time spent in the target quadrant by each group of 4-week-old mice (Ttest=0.21943). (C) Graph showing the latency period for 10-week-old exercising mice to reach the platform per day as compared to their sedentary group. Statistical significance was measured by unpaired t-test *p<0.05. (D) Graph indicating the average time spent in the target quadrant by each group of 10-week-old mice (Ttest=0.17344). (E) Graph showing the latency period for 22-week-old exercising mice to reach the platform per day as compared to their sedentary group. Statistical significance was measured by unpaired t-test **p<0.01. (F) Graph indicating the average time spent in the target quadrant by each group of 22-week-old mice (Ttest=0.22147).

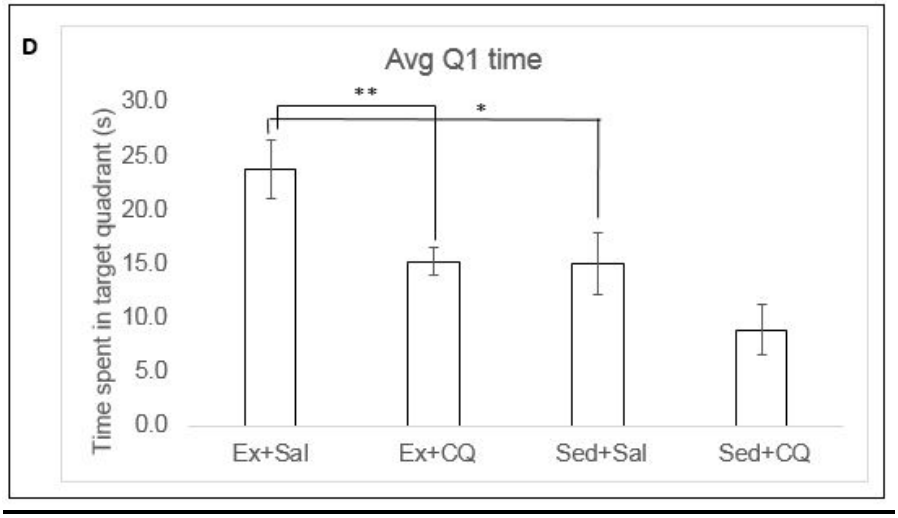
3.3 Inhibition of autophagy decreases learning and memory formation in 10-week-old mice, but has no effect on 4 and 32-week-old mice

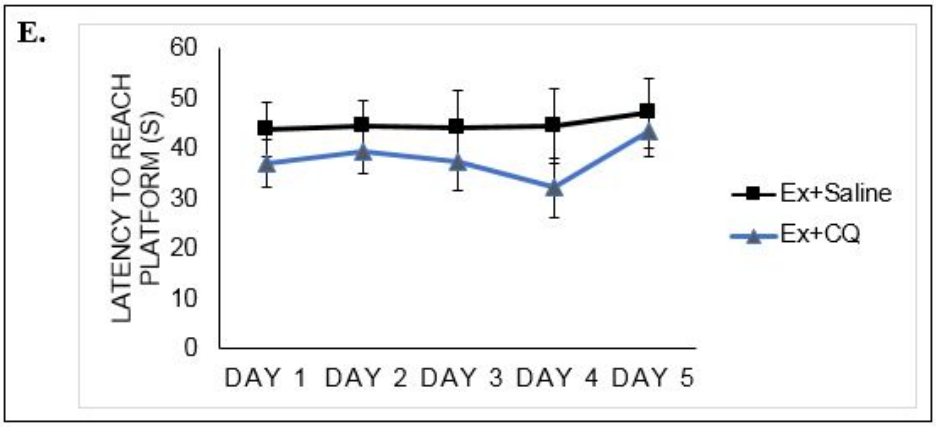
Having showed that short-term exercise induces autophagy and enhances learning and memory formation in mature adult mice, we sought to determine whether the induction of autophagy is linked to enhanced learning and memory formation. As a result, we inhibited exercise-induced autophagy using a brain-permeable inhibitor, chloroquine diphosphate, and determined whether this inhibition affects learning and memory formation in the different age groups. We used mice that have undergone the short-term exercise paradigm and injected them with chloroquine diphosphate as soon as they began the MWM training. We administered the same dose every day until the final harvest. In 4-week-old mice, chloroquine diphosphate did not impact learning and memory formation, neither in terms of latency to reach the platform during the training phase (Figure 7A), nor in terms of time spent in the target quadrant when memory formation was tested (Figure 7B). In 10-week-old mice, we observed that exercise enhanced learning by decreasing the escape latency time. Interestingly, inhibition of autophagy by chloroquine diphosphate abolished the exercise induced learning (Figure 7C). Moreover, exercise enhanced memory formation in the 10-week-old mice whereas inhibition of autophagy by chloroquine diphosphate abolished this exercise induced effect (Ttest= 0.0085) (Figure 7D). These results are consistent with our observation in figure 5C, where we saw that exercise enhanced autophagy in this mice age group. Considering that this form of spatial memory is hippocampus-dependent, it makes sense that we observe that the inhibition of autophagy has negative effects on the learning and memory formation in 10-week-old mice, but not 4-week-old mice. Middle age mice (32-weeks-old) showed opposite results where learning showed slight but not significant improvement (Figure 7E) and memory performance was enhanced (Figure 7F) upon chloroquine injections (Ttest= 0.01396).


A
Latency to reach platform (s)
70
60
50
40
30
20
10
0
Day 1
Day 2
Day 3
Day 4
Day 5
Ex+saline
Ex+CQ
Sed+CQ
Sed+Sal


B
Average Q1 time
Time spent in target quadrant (s)
40.0
30.0
20.0
10.0
0.0
Ex+Sal
Ex+CQ
Sed+CQ
Sed+Sal


C
Latency to reach platform (s)
70
60
50
40
30
20
10
0
*
**
**
*
***
Day 1
Day 2
Day 3
Day 4
Day 5
Ex+Sal
Ex+CQ
Sed+Sal
Sed+CQ


D
Avg Q1 time
Time spent in target quadrant (s)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
**
*
Ex+Sal
Ex+CQ
Sed+Sal
Sed+CQ


E.
LATENCY TO REACH PLATFORM (S)
60
50
40
30
20
10
0
DAY 1 DAY 2 DAY 3 DAY 4 DAY 5
Ex+Saline
Ex+CQ

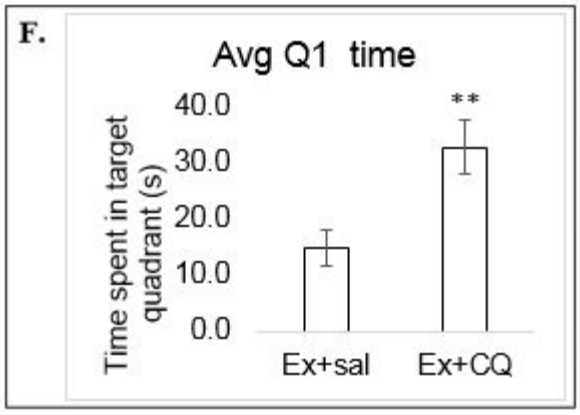

F.
Avg Q1 time
Time spent in target quadrant (s)
40.0
30.0
20.0
10.0
0.0
**
Ex+sal
Ex+CQ

Figure 7: Inhibition of autophagy during training in the Morris Water Maze affects performance differently according to age.

(A) Graph showing the latency period for 4-week-old mice to reach the platform per day comparing exercising mice taking saline injections to ones taking injections of the autophagy inhibitor chloroquine (CQ) as well as to a group of sedentary mice being administered the inhibitor CQ. (B) Graph indicating the average time spent in the target quadrant by each group of 4-week-old mice. (C) Graph showing the latency period for 10-week-old mice to reach the platform per day comparing exercising mice taking saline injections to ones taking injections of the inhibitor CQ as well as to a group of sedentary mice being administered either saline or the inhibitor CQ. Statistical significance from day 1 to 5 between Ex+Sal and Sed+Sal groups was measured by unpaired t-test *$p<0.05$, **$p<0.01$, and ***$p<0.001$ as well as between Ex+CQ and Sed+Sal group *$p<0.05$. (D) Graph indicating the average time spent in the target quadrant by each group of 10-week-old mice. Statistical significance between Ex+Sal and Ex+CQ groups was measured by unpaired t-test **$p<0.01$ and *$p<0.05$ between Ex+Sal and Sed+Sal. (E) Graph showing the latency period for 22 to 32-week-old mice to reach the platform per day comparing exercising mice taking saline injections to ones taking injections of the inhibitor CQ. (F) Graph indicating the average time spent in the target quadrant by each group of 32-week-old mice. Statistical significance was measured by unpaired t-test **$p<0.01$.

3.4. Inhibition of autophagy negatively affects BDNF levels in the hippocampus of 10 week old mice

Since inhibition of autophagy lead to a decline in cognitive performance in 10-week-old mice as seen in the previous MWM results (Figures 7C and D), we hypothesized that autophagy regulates BDNF levels in the hippocampus to enhance learning and improve memory formation. We used the hippocampus of 10-week-old mice that were either treated with saline or chloroquine diphosphate during the behavioral tests, extracted proteins, and performed a western blot. Our results indicated that indeed BDNF levels decrease (Ttest=0.08247) when autophagy is inhibited (Figure 8A and B). We are currently increasing the n number for these experiments.

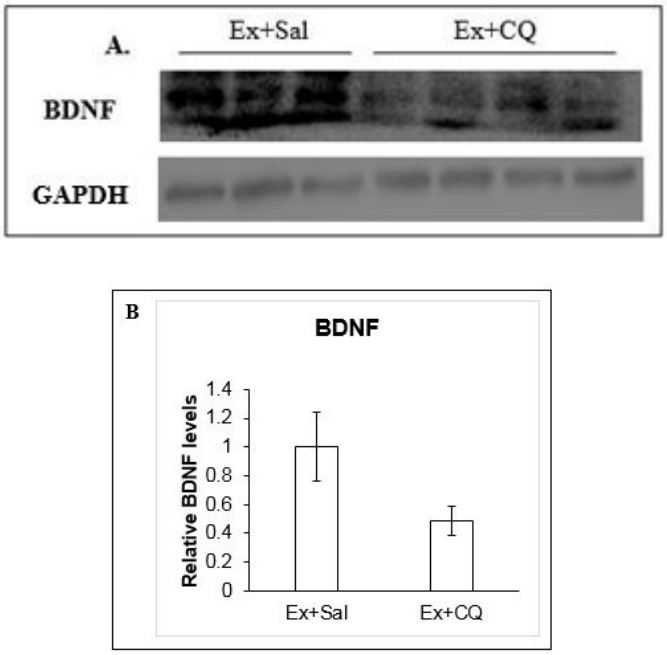


Figure 8: BDNF levels decrease in the hippocampus of 10-week-old exercising mice upon autophagy inhibition.

(A) Representative western blot image depicting the levels of BDNF in exercising mice that were administered the autophagy inhibitor chloroquine (CQ) (first 3 lanes) as compared to the control group of exercising mice that only took the vehicle saline (Sal) (last 4 lanes) along with the loading control GAPDH. (B) Relative quantification of BDNF western blot.

Chapter Four

Discussion

Researchers have long been interested in studying the positive effects of exercise on the body, and specifically how it affects the brain and cognitive function. It was demonstrated that physical activity increases neurotransmitter production, growth factor release, synaptic plasticity, spine density, angiogenesis and neurogenesis, as well as learning and memory and resilience to depression and anxiety (van Praag, Christie, Sejnowski and Gage, 1999; van Praag, 2008; Lawlor, 2001; Cotman, Berchtold and Christie, 2007 ; van Praag, 2009). As people age, they acquire an increased risk of developing neurodegenerative diseases (Daniele, Giacomelli and Martini, 2018) such as Alzheimer's or Parkinson's disease. As a result, their cognitive functions deteriorate and neurogenesis and synaptic processes decline (Kuhn, Dickinson-Anson and Gage, 1996 ; Barnes, 1994). However, studies have shown that exercise has the ability to reverse the negative effects of aging on the brain (van Praag, 2005; Wong-Goodrich et al., 2010). In our study, we focused on determining a mechanism through which voluntary exercise impacts learning and memory formation. Several studies have established that upon physical activity, hippocampal *bdnf* expression levels are increased (Neeper, Góauctemez-Pinilla, Choi and Cotman, 1995 ; Oliff, Berchtold, Isackson and Cotman, 1998) to induce learning and memory formation (Brigadski and Leßmann, 2014 ; Vaynman, Ying and Gomez-Pinilla, 2004).

Diet, like exercise, has a major impact on brain function. For instance, our lab has recently shown that a high protein diet promotes resilience to chronic social defeat stress (Nasrallah et al., 2019). Others have shown that caloric restriction activates hippocampal BDNF signaling and a downstream PI3K/Akt pathway to inhibit the transcription of autophagy related genes and enhanced memory (Nikoletopoulou et al., 2017). Therefore, since the signaling pathways activated by dietary restriction are usually similar to those induced by exercise, we became interested in determining

whether autophagy is involved in exercise-induced learning and memory formation; and if there is an age-specific role.

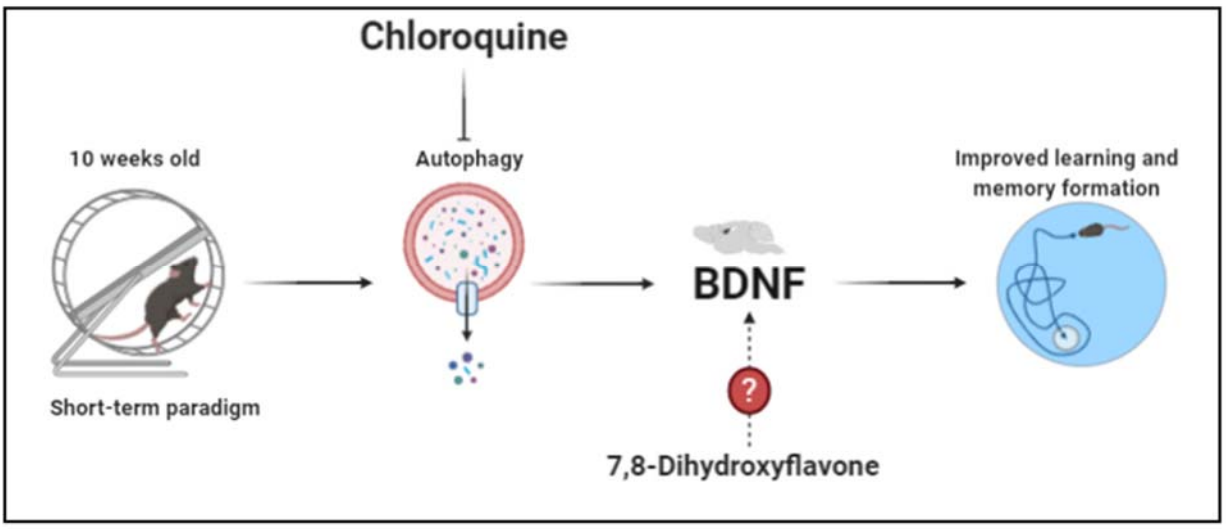
In order to test our hypothesis, we first wanted to verify whether voluntary wheel-running induces an increase in autophagy in the mouse brain. Some studies had previously shown that autophagy is activated in the exercising skeletal muscle and even in the brain (Brandt, Gunnarsson, Bangsbo and Pilegaard, 2018. ; Taylor & Francis, 2012). We decided to implement a short-term exercise paradigm that consists of 2 weeks of voluntary running and then looked at autophagy markers to determine if there was an effect on that process. To assess age-related effects, we mainly focused on 3 different age groups: juvenile mice that are 4-weeks-old, middle age mice that are 22 or 32-weeks-old, as well as an intermediate group of adult mice that are 10-weeks-old. By looking at the protein levels of light chain LC3B right after this exercise paradigm, our results indicated that autophagy is induced in the cortex of young mice and then this induction shifts to the hippocampus in 10-week-old ones, only to disappear completely in both brain regions of older mice. This points out that there is an induction of autophagy in the brain and that it is age-dependent.

We next assessed the behavioral aspects by allowing the mice to train in the MWM. This test assesses hippocampal-dependent spatial learning and memory. To understand how short-term exercise affects learning and memory formation, we measured the escape latency period, the time the mouse needs to reach the platform during the learning phase that consists of 5 days. The mouse is said to be learning better if it has a shorter escape latency period. On the probe trial/memory test day, the time spent in the target quadrant (Q1) that previously contained the platform is measured. The more time the mouse spends in Q1 searching for the platform, the better its memory. Our results on the 4-week-old mice indicate that short term exercise enhances learning, but does not have significant effects on memory formation. Our results on the10-week-old mice verify that learning and memory are enhanced with short-term voluntary exercise.

We, therefore, raised the question of whether autophagy might be involved in mediating the beneficial effects of exercise on cognitive performance. In order to assess that, we hypothesized that if autophagy was inhibited after short-term exercise, exercise-induced learning and memory formation would be negatively affected, specifically in 10 week-old mice. As a result, after short-term voluntary wheel running, we started treating the mice with chloroquine diphosphate, an autophagy inhibitor, at a dose of 50mg/kg daily all throughout the 8 days of training and memory tests. Indeed, the results verified our hypothesis since exercise-induced spatial learning and memory formation are attenuated in 10-week-old exercising mice that were treated with chloroquine as compared to ones treated with the vehicle (saline). Interestingly, autophagy inhibition did not affect learning and memory formation in the juvenile mice. This is consistent with our data that exercise does not induce autophagy marker protein levels in the hippocampus of these mice. Moreover, when we assessed the effect of autophagy on middle-aged mice, we observed that inhibition of autophagy enhances exercise-induced learning and memory formation. This is an unexpected result as we could not observe significant regulation of autophagy markers in this mouse group (Figures 7E and F). We are currently exploring whether exercise affects other autophagy markers in this age group including the autophagy proteins. Our results suggest that exercise differentially regulates autophagy in an age-dependent manner. In addition, they also suggest that autophagy itself differentially modulates hippocampal-dependent learning and memory formation in an age-dependent manner.

We next wanted to test whether exercise-mediated autophagy in 10-week-old mice induces BDNF to promote learning and memory. We performed a western blot and looked at the protein levels of hippocampal BDNF in a group of exercising mice treated with saline versus a group treated with chloroquine. The data was consistent with our hypothesis that BDNF induction is downstream of autophagy when exercise stimulates spatial learning and memory formation. We are currently working in increasing the n number for these experiments.

In this study, we provided evidence that in a short-term voluntary exercise paradigm, autophagy is induced in the hippocampus of 10-week-old mice, activating BDNF to promote spatial learning and memory formation (Figure 9). For future studies, it would be interesting to look at whether activating BDNF with a TrkB activator such as 7,8-dihydroxyflavone can bypass the inhibition of autophagy.



<u>Figure 9</u>: A proposed model by which short-term exercise promotes learning and memory formation.

In 10 week old mice, a short-term paradigm of voluntary wheel running was shown to induce autophagy in hippocampal tissues. Exercise-induced autophagy activates BDNF in the hippocampus, thus promoting learning and memory. Inhibition of autophagy by chloroquine during behavioral training demonstrated deficits in learning and memory performance of exercising 10-week-old mice. We hypothesize that using 7,8-dihydroxyflavone to activate BDNF can bypass autophagy inhibition.

References

Adlard, P., 2005. *Voluntary Exercise Decreases Amyloid Load In A Transgenic Model Of Alzheimer's Disease*.

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K. and Timmusk, T., 2007. *Mouse And Ratbdnf Gene Structure And Expression Revisited*.

Alirezaei, M., Kemball, C., Flynn, C., Wood, M., Whitton, J. and Kiosses, W., 2010. *Short-Term Fasting Induces Profound Neuronal Autophagy*.

Autry, A. and Monteggia, L., 2012. *Brain-Derived Neurotrophic Factor And Neuropsychiatric Disorders*.

Barmada, S., Serio, A., Arjun, A., Bilican, B., Daub, A., Ando, D., Tsvetkov, A., Pleiss, M., Li, X., Peisach, D., Shaw, C., Chandran, S. and Finkbeiner, S., 2014. *Autophagy Induction Enhances TDP43 Turnover And Survival In Neuronal ALS Models*.

Barnes, C., 1994. *Normal Aging: Regionally Specific Changes In Hippocampal Synaptic Transmission*.

Berchtold, N., Castello, N. and Cotman, C., 2010. *Exercise And Time-Dependent Benefits To Learning And Memory*.

Bettio, L., Thacker, J., Hutton, C. and Christie, B., 2019. *Modulation Of Synaptic Plasticity By Exercise*.

Brandt, N., Gunnarsson, T., Bangsbo, J. and Pilegaard, H., 2018. *Exercise And Exercise Training-Induced Increase In Autophagy Markers In Human Skeletal Muscle*.

Brigadski, T. and Leßmann, V., 2014. *BDNF: A Regulator Of Learning And Memory Processes With Clinical Potential*.

Chieffi, S., Messina, G., Villano, I., Messina, A., Valenzano, A., Moscatelli, F., Salerno, M., Sullo, A., Avola, R., Monda, V., Cibelli, G. and Monda, M., 2017. *Neuroprotective Effects Of Physical Activity: Evidence From Human And Animal Studies*.

Cirulli, F., Berry, A., Chiarotti, F. and Alleva, E., 2004. *Intrahippocampal Administration Of BDNF In Adult Rats Affects Short-Term Behavioral Plasticity In The Morris Water Maze And Performance In The Elevated Plus-Maze*.

Cotman, C., Berchtold, N. and Christie, L., 2007. *Exercise Builds Brain Health: Key Roles Of Growth Factor Cascades And Inflammation*.Daniele, S., Giacomelli, C. and Martini, C., 2018. *Brain Ageing And Neurodegenerative Disease: The Role Of Cellular Waste Management*.

Daniele, S., Giacomelli, C. and Martini, C., 2018. *Brain Ageing And Neurodegenerative Disease: The Role Of Cellular Waste Management*.

Dhaliwal, J., Trinkle-Mulcahy, L. and Lagace, D., 2017. *Autophagy And Adult Neurogenesis: Discoveries Made Half A Century Ago Yet In Their Infancy Of Being Connected*.

Diederich, K., Bastl, A., Wersching, H., Teuber, A., Strecker, J., Schmidt, A., Minnerup, J. and Schäbitz, W., 2017. *Effects Of Different Exercise Strategies And Intensities On Memory Performance And Neurogenesis*.

Ding, Q., Vaynman, S., Akhavan, M., Ying, Z. and Gomez-Pinilla, F., 2006. *Insulin-Like Growth Factor I Interfaces With Brain-Derived Neurotrophic Factor-Mediated Synaptic Plasticity To Modulate Aspects Of Exercise-Induced Cognitive Function*.

Duman, C., Schlesinger, L., Russell, D. and Duman, R., 2008. *Voluntary Exercise Produces Antidepressant And Anxiolytic Behavioral Effects In Mice*.

El Hayek, L., Khalifeh, M., Zibara, V., Abi Assaad, R., Emmanuel, N., Karnib, N., El-Ghandour, R., Nasrallah, P., Bilen, M., Ibrahim, P., Younes, J., Abou Haidar, E., Barmo, N., Jabre, V., Stephan, J. and Sleiman, S., 2019. *Lactate Mediates The Effects Of Exercise On Learning And Memory Through SIRT1-Dependent Activation Of Hippocampal Brain-Derived Neurotrophic Factor (BDNF)*.

Eric E Essick, F., 2010. *Oxidative Stress And Autophagy In Cardiac Disease, Neurological Disorders, Aging And Cancer*. [online] PubMed Central (PMC). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952075/>.Erickson, K., Miller, D. and Roecklein, K., 2012. *The Aging Hippocampus*.

Fabel, K., Fabel, K., Tam, B., Kaufer, D., Baiker, A., Simmons, N., Kuo, C. and Palmer, T., 2003. *VEGF Is Necessary For Exercise-Induced Adult Hippocampal Neurogenesis*.

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F. and Christie, B., 2004. *Effects Of Voluntary Exercise On Synaptic Plasticity And Gene Expression In The Dentate Gyrus Of Adult Male Sprague–Dawley Rats In Vivo*.

Frake, R., Ricketts, T., Menzies, F. and Rubinsztein, D., 2015. *Autophagy And Neurodegeneration*.

Frazzitta, G., Balbi, P., Maestri, R., Bertotti, G., Boveri, N. and Pezzoli, G., 2013. *The Beneficial Role Of Intensive Exercise On Parkinson Disease Progression*.

Garatachea, N., Pareja-Galeano, H., Sanchis-Gomar, F., Santos-Lozano, A., Fiuza-Luces, C., Morán, M., Emanuele, E., Joyner, M. and Lucia, A., 2015. *Exercise Attenuates The Major Hallmarks Of Aging*.

Glatigny, M., Moriceau, S., Rivagorda, M., Ramos-Brossier, M., Nascimbeni, A., Lante, F., Shanley, M., Boudarene, N., Rousseaud, A., Friedman, A., Settembre, C., Kuperwasser, N., Friedlander, G., Buisson, A., Morel, E., Codogno, P. and Oury, F., 2019. *Autophagy Is Required For Memory Formation And Reverses Age-Related Memory Decline*.

Glick, D., Barth, S. and Macleod, K., 2010. *Autophagy: Cellular And Molecular Mechanisms*.

Gomez-Pinilla, F., Vaynman, S. and Ying, Z., 2010. *Brain-Derived Neurotrophic Factor Functions As A Metabotrophin To Mediate The Effects Of Exercise On Cognition*.

Hallböök, F., 1999. *Evolution Of The Vertebrate Neurotrophin And Trk Receptor Gene Families*.

Halling, J. and Pilegaard, H., 2017. *Autophagy-Dependent Beneficial Effects Of Exercise*.

He, C., Sumpter, Jr., R. and Levine, B., 2012. *Exercise Induces Autophagy In Peripheral Tissues And In The Brain*.

He, L., Lu, J. and Yue, Z., 2013. *Autophagy In Ageing And Ageing-Associated Diseases*.

Huang, E. and Reichardt, L., 2001. *Neurotrophins: Roles In Neuronal Development And Function*.

Hylin, M., Zhao, J., Tangavelou, K., Rozas, N., Hood, K., MacGowan, J., Moore, A. and Dash, P., 2017. *A Role For Autophagy In Long-Term Spatial Memory Formation In Male Rodents*.

Jackson, S., Andrews, N., Ball, D., Bellantuono, I., Gray, J., Hachoumi, L., Holmes, A., Latcham, J., Petrie, A., Potter, P., Rice, A., Ritchie, A., Stewart, M., Strepka, C., Yeoman, M. and Chapman, K., 2017. *Does Age Matter? The Impact Of Rodent Age On Study Outcomes*.

Khrimian, L., Obri, A., Ramos-Brossier, M., Rousseaud, A., Moriceau, S., Nicot, A., Mera, P., Kosmidis, S., Karnavas, T., Saudou, F., Gao, X., Oury, F., Kandel, E. and Karsenty, G., 2017. *Gpr158 Mediates Osteocalcin'S Regulation Of Cognition*.

Kosmidis, S., Polyzos, A., Harvey, L., Youssef, M., Denny, C., Dranovsky, A. and Kandel, E., 2018. *Rbap48 Protein Is A Critical Component Of GPR158/OCN Signaling And Ameliorates Age-Related Memory Loss*.

Kowiański, P., Lietzau, G., Czuba, E., Waśkow, M., Steliga, A. and Moryś, J., 2017. *BDNF: A Key Factor With Multipotent Impact On Brain Signaling And Synaptic Plasticity*.

Kuhn, H., Dickinson-Anson, H. and Gage, F., 1996. *Neurogenesis In The Dentate Gyrus Of The Adult Rat: Age-Related Decrease Of Neuronal Progenitor Proliferation*.

Kulkarni, A., Chen, J. and Maday, S., 2018. *Neuronal Autophagy And Intercellular Regulation Of Homeostasis In The Brain*.

Law, L., Rol, R., Schultz, S., Dougherty, R., Edwards, D., Koscik, R., Gallagher, C., Carlsson, C., Bendlin, B., Zetterberg, H., Blennow, K., Asthana, S., Sager, M., Hermann, B., Johnson, S., Cook, D. and Okonkwo, O., 2018. *Moderate Intensity Physical Activity Associates With CSF Biomarkers In A Cohort At Risk For Alzheimer's Disease*.

Lawlor, D., 2001. *The Effectiveness Of Exercise As An Intervention In The Management Of Depression: Systematic Review And Meta-Regression Analysis Of Randomised Controlled Trials*. Lewin, G. and Barde, Y., 1996. *Physiology Of The Neurotrophins*.

Liang, Y., 2019. *Emerging Concepts And Functions Of Autophagy As A Regulator Of Synaptic Components And Plasticity*.

Longo, V. and Mattson, M., 2014. *Fasting: Molecular Mechanisms And Clinical Applications*.

López-Otín, C., Blasco, M., Partridge, L., Serrano, M. and Kroemer, G., 2013. *The Hallmarks Of Aging*.

Lubin, F., Roth, T. and Sweatt, J., 2008. *Epigenetic Regulation Of Bdnf Gene Transcription In The Consolidation Of Fear Memory*.

Mera, P., Laue, K., Ferron, M., Confavreux, C., Wei, J., Galán-Díez, M., Lacampagne, A., Mitchell, S., Mattison, J., Chen, Y., Bacchetta, J., Szulc, P., Kitsis, R., de Cabo, R., Friedman, R., Torsitano, C., McGraw, T., Puchowicz, M., Kurland, I. and Karsenty, G., 2016. *Osteocalcin Signaling In Myofibers Is Necessary And Sufficient For Optimum Adaptation To Exercise*.

Metaxakis, A., Ploumi, C. and Tavernarakis, N., 2018. *Autophagy In Age-Associated Neurodegeneration*.

Miranda, M., Morici, J., Zanoni, M. and Bekinschtein, P., 2019. *Brain-Derived Neurotrophic Factor: A Key Molecule For Memory In The Healthy And The Pathological Brain*.

Mitre, M., Mariga, A. and Chao, M., 2017. *Neurotrophin Signalling: Novel Insights Into Mechanisms And Pathophysiology*.

Mizushima, N. and Komatsu, M., 2011. *Autophagy: Renovation Of Cells And Tissues*.

Morris, R., 1984. *Developments Of A Water-Maze Procedure For Studying Spatial Learning In The Rat*.

Moscat, J. and Diaz-Meco, M., 2009. *P62 At The Crossroads Of Autophagy, Apoptosis, And Cancer*.

Mul, J., Soto, M., Cahill, M., Ryan, R., Takahashi, H., So, K., Zheng, J., Croote, D., Hirshman, M., la Fleur, S., Nestler, E. and Goodyear, L., 2018. *Voluntary Wheel Running Promotes Resilience To Chronic Social Defeat Stress In Mice: A Role For Nucleus Accumbens Δfosb*.

Nasrallah, P., Haidar, E., Stephan, J., El Hayek, L., Karnib, N., Khalifeh, M., Barmo, N., Jabre, V., Houbeika, R., Ghanem, A., Nasser, J., Zeeni, N., Bassil, M. and Sleiman, S., 2019. *Branched-Chain Amino Acids Mediate Resilience To Chronic Social Defeat Stress By Activating BDNF/TRKB Signaling*.

Neeper, S., Góauctemez-Pinilla, F., Choi, J. and Cotman, C., 1995. *Exercise And Brain Neurotrophins*.

Nikoletopoulou, V., Sidiropoulou, K., Kallergi, E., Dalezios, Y. and Tavernarakis, N., 2017. *Modulation Of Autophagy By BDNF Underlies Synaptic Plasticity*.

Obri, A., Khrimian, L., Karsenty, G. and Oury, F., 2018. *Osteocalcin In The Brain: From Embryonic Development To Age-Related Decline In Cognition*.

Oliff, H., Berchtold, N., Isackson, P. and Cotman, C., 1998. *Exercise-Induced Regulation Of Brain-Derived Neurotrophic Factor (BDNF) Transcripts In The Rat Hippocampus*.

Patki, G., Solanki, N., Atrooz, F., Ansari, A., Allam, F., Jannise, B., Maturi, J. and Salim, S., 2014. *Novel Mechanistic Insights Into Treadmill Exercise Based Rescue Of Social Defeat-Induced Anxiety-Like Behavior And Memory Impairment In Rats*.

Phillips, C., 2017. *Brain-Derived Neurotrophic Factor, Depression, And Physical Activity: Making The Neuroplastic Connection*.

Phillips, C., Baktir, M., Srivatsan, M. and Salehi, A., 2014. *Neuroprotective Effects Of Physical Activity On The Brain: A Closer Look At Trophic Factor Signaling*.

Piepmeier, A. and Etnier, J., 2015. *Brain-Derived Neurotrophic Factor (BDNF) As A Potential Mechanism Of The Effects Of Acute Exercise On Cognitive Performance*.

Radak, Z., Hart, N., Sarga, L., Koltai, E., Atalay, M., Ohno, H. and Boldogh, I., 2010. *Exercise Plays A Preventive Role Against Alzheimer's Disease*.

Rocchi, A. and He, C., 2017. *Activating Autophagy By Aerobic Exercise In Mice*.

Rosas-Vidal, L., Do-Monte, F., Sotres-Bayon, F. and Quirk, G., 2014. *Hippocampal–Prefrontal BDNF And Memory For Fear Extinction*.

Rubinsztein, D., Mariño, G. and Kroemer, G., 2011. *Autophagy And Aging*.

Schwalm, C., Jamart, C., Benoit, N., Naslain, D., Prémont, C., Prévet, J., Van Thienen, R., Deldicque, L. and Francaux, M., 2015. *Activation Of Autophagy In Human Skeletal Muscle Is Dependent On Exercise Intensity And AMPK Activation*.

Segal, R., 2003. *SELECTIVITY IN NEUROTROPHIN SIGNALING: Theme And Variations*. [online] Annual Reviews. Available at: <https://www.annualreviews.org/doi/full/10.1146/annurev.neuro.26.041002.131421?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed>.

Shan, C., Ghosh, A., Guo, X., Wang, S., Hou, Y., Li, S. and Liu, J., 2019. *Roles For Osteocalcin In Brain Signalling: Implications In Cognition- And Motor-Related Disorders*.

Sleiman, S., 2016. *Exercise Promotes The Expression Of Brain Derived Neurotrophic Factor (BDNF) Through The Action Of The Ketone Body B-Hydroxybutyrate*. [online] eLife. Available at: <https://doi.org/10.7554/eLife.15092.001>.

Tabuchi, A., Sakaya, H., Kisukeda, T., Fushiki, H. and Tsuda, M., 2002. *Involvement Of An Upstream Stimulatory Factor As Well As Camp-Responsive Element-Binding Protein In The Activation Of Brain-Derived Neurotrophic Factor Gene Promoter I*.

Tajiri, N., Yasuhara, T., Shingo, T., Kondo, A., Yuan, W., Kadota, T., Wang, F., Baba, T., Tayra, J., Morimoto, T., Jing, M., Kikuchi, Y., Kuramoto, S., Agari, T., Miyoshi, Y., Fujino, H., Obata, F., Takeda, I., Furuta, T. and Date, I., 2010. *Exercise Exerts Neuroprotective Effects On Parkinson's Disease Model Of Rats*.

Taylor & Francis. 2012. *Exercise Induces Autophagy In Peripheral Tissues And In The Brain*. [online] Available at: <https://www.tandfonline.com/doi/full/10.4161/auto.21327?scroll=top&needAccess=true>.

Towers, C. and Thorburn, A., 2016. *Therapeutic Targeting Of Autophagy*.

Trejo, J., Carro, E. and Torres-Alemán, I., 2001. *Circulating Insulin-Like Growth Factor I Mediates Exercise-Induced Increases In The Number Of New Neurons In The Adult Hippocampus*.

Tripathi, T., 2019. *Autophagy Promotes Memory Formation*. [online] Pubs.acs.org. Available at: <https://pubs.acs.org/doi/10.1021/acschemneuro.9b00317>.

van Praag, H., 2005. *Exercise Enhances Learning And Hippocampal Neurogenesis In Aged Mice*.

van Praag, H., 2008. *Neurogenesis And Exercise: Past And Future Directions*.

van Praag, H., 2009. *Exercise And The Brain: Something To Chew On*.

van Praag, H., Christie, B., Sejnowski, T. and Gage, F., 1999. *Running Enhances Neurogenesis, Learning, And Long-Term Potentiation In Mice*.

Vaynman, S., Ying, Z. and Gomez-Pinilla, F., 2004. *Hippocampal BDNF Mediates The Efficacy Of Exercise On Synaptic Plasticity And Cognition*.

Vaynman, S., Ying, Z., Yin, D. and Gomez-Pinilla, F., 2006. *Exercise Differentially Regulates Synaptic Proteins Associated To The Function Of BDNF*.

Wong-Goodrich, S., Pfau, M., Flores, C., Fraser, J., Williams, C. and Jones, L., 2010. *Voluntary Running Prevents Progressive Memory Decline And Increases Adult Hippocampal Neurogenesis And Growth Factor Expression After Whole-Brain Irradiation*.

Wrann, C., White, J., Salogiannnis, J., Laznik-Bogoslavski, D., Wu, J., Ma, D., Lin, J., Greenberg, M. and Spiegelman, B., 2013. *Exercise Induces Hippocampal BDNF Through A PGC-1A/FNDC5 Pathway*.

Xing, Y., Yang, S., Wang, M., Feng, Y., Dong, F. and Zhang, F., 2019. *The Beneficial Roles Of Exercise Training Via Autophagy In Neurological Diseases And Possible Mechanisms*.

Yamada, K., Mizuno, M. and Nabeshima, T., 2002. *Role For Brain-Derived Neurotrophic Factor In Learning And Memory*.

Zatloukal, K., Stumptner, C., Fuchsbichler, A., Heid, H., Schnoelzer, M., Kenner, L., Kleinert, R., Prinz, M., Aguzzi, A. and Denk, H., 2002. *P62 Is A Common Component Of Cytoplasmic Inclusions In Protein Aggregation Diseases*.

Zhang, J., He, Z., Wang, L., Yuan, W., Li, L., Hou, W., Yang, Y., Guo, Q., Zhang, X., Cai, W., An, S. and Tai, F., 2019. *Voluntary Wheel Running Reverses Deficits In Social Behavior Induced By Chronic Social Defeat Stress In Mice: Involvement Of The Dopamine System*.

Zhao, N., Zhang, X., Song, C., Yang, Y., He, B. and Xu, B., 2018. *The Effects Of Treadmill Exercise On Autophagy In Hippocampus Of APP/PS1 Transgenic Mice*.