THE NEUROPROTECTIVE EFFECTS OF MODERATE TREADMILL EXERCISE IN A RAT MODEL OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss, spatial disorientation, and aberrant behaviors. The most important risk factor of AD is aging. Accumulating evidence suggests a neuroprotective role of regular exercise in aging associated memory impairment. In this study, we investigated the ability of regular moderate treadmill exercise to prevent impairment of cognitive and non-cognitive functions, long-term potentiation (LTP), and related signaling pathways in a rat model of AD, which was achieved by i.c.v. infusion of $A\beta_{1-42}$ peptides (250 pmol/day for 2 weeks). We utilized behavioral assessment, *in vivo* electrophysiological recording, and immunoblotting in 4 groups of adult Wistar rats: control, treadmill exercise (Ex), β-amyloid-infused (Aβ), and amyloid-infused/treadmill exercised (Ex/Aβ). Our findings indicated that Aβ rats exhibited impaired spatial learning and memory as tested in the radial arm water maze (RAWM). Compared to all other groups, these rats also displayed increased anxiety-like behaviors as indicated by less time spent in the center area of the open field apparatus and the elevated plus maze (EPM), more time in the dark area of the light-dark box, and longer time in the closed arms of the EPM paradigm. Extracellular recordings in urethane-anesthetized rats revealed that these amyloid-infused animals showed suppressed early phase (E-) and late phase (L-) LTP in both CA1 and DG areas, which correlated with deficient signaling pathways in these two brain regions. For example, Western blot analysis indicated that Aβ rats exhibited deleterious alterations in the levels of AD- and LTP-related molecules including amyloid precursor protein (APP), β-secretase enzyme (BACE-1), calcineurin (PP2B), brain derived-

neurotrophic factor (BDNF), Ca^{2+}/calmodulin dependent protein kinases II and IV (CaMKII and CaMKIV), cAMP response element binding protein (CREB), and extracellular signal-regulated kinase 1/2 (ERK1/2). Compared to controls, Ex and Ex/Aβ rats showed a similar behavioral performance with normal hippocampal LTP and no detrimental changes in the levels of those LTP- and memory-related molecules in both areas. Thus, regular moderate treadmill exercise may be beneficial in preserving cognitive and non-cognitive functions in the AD brains by preventing the detrimental effects of amyloid toxicity on the synapses and key signaling pathways.

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ABBREVIATIONS

5HT- Serotonin

Aβ- amyloid protein

ACh- acetylcholine

AD- Alzheimer's disease

AMPA- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

AP5- 2-amino-5-phosphonopentanoate

ATP- Adenosine triphosphate

BDNF- Brain-derived neurotrophic factor

CA (1-3)-Cornu Ammonis (1-3)

Ca^{2+}- Calcium

CaM- calmodulin

Ca/CaM- Calcium- calmodulin

CaMKII- Calcium-calmodulin protein kinase II

CaMKIV- Calcium-calmodulin protein kinase IV

cAMP- Cyclic adenosine monophosphate

CNS- central nervous system

CREB- cAMP response element binding protein

CSF- cerebrospinal fluid

DG- Dentate gyrus

E-LTP- early phase LTP

Ex- exercise

fEPSP- field excitatory post-synaptic potential

GDNF- glial cell line neurotrophic factor

HFS- high frequency stimulation

I/O- input/output

I.p.-intra-peritoneally

LTD- Long term depression

L-LTP- late phase LTP

LTP- Long term potentiation

MAPK/ERK- mitogen activated protein kinase/ extracellular regulated kinase

MHFS- multiple high frequency stimulation

MWM- Morris water maze

NE- norepinephrine

NFκB- nuclear factor kappa beta

NGF- neurotrophic growth factor

NMDA- N-Methyl-D-aspartic acid receptor

PKA- Protein kinase A/ cAMP dependent protein kinase

PNS- peripheral nervous system

PP- Perforant path

PP2B – Protein phosphatase 2B/ Calcineurin

pSpike- population spike

RAM- Radial arm maze

RAWM- Radial arm water maze

TrkB- tyrosine kinase B receptor

VEGF- Vascular endothelial growth factor

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1. INTRODUCTION AND STATEMENT OF THE PROBLEM

Alzheimer's disease (AD), a leading cause of dementia in the elderly, is an irreversible neurodegenerative disorder in which patients experience a progressive memory loss in addition to psychopathic behaviors. The increasing life expectancy not only challenges the individual's later life but also places heavy caregiving burden and spiraling financial cost on family and society. In 2006, the number of people suffering from AD globally is approximately 26.6 million and this is expected to increase to 106.8 million in 2050 (Brookmeyer et al., 2007). According to the Alzheimer's Association, AD is the sixth leading cause in the United States where the mortality rate resulting from AD has tremendously increased (about 69%) during the last decade compared to other causes of death (e.g. cancer, heart diseases). One of the current crises that concern all people including Americans is the retirement plan for older people. Aside from substantial venue for assisted care living, demented elderly require an extremely high-level of care and close supervision. As the first baby boomer reached 65th birthday on January 1st, 2011 and the cost of health care is on the rise, America faces a serious challenge providing financial assistance for these millions of people. There will be approximately 10 million baby boomers who will have AD. This will cost the nation $20 trillion, which will be enough to pay off the nation debt and still send a $20,000 check to every adult in America (Alzheimer's Association). Not only AD patients suffer the ravaging consequences of the pathology, but their family members also undergo the prolonged agony: to cope with the disease manifestations and to witness the death of their loved ones.

The pathogenesis of AD has been a puzzle to scientists since its first clinical descriptions by the physician Alois Alzheimer in 1906. Many hypotheses have been postulated but none is able to account for the full-blown spectrum of cognitive and non-cognitive disturbances associated with the disease. AD patients experience progressive memory loss accompanied by non-cognitive deficits (e.g. anxiety, irritability, aggression), which greatly reduce their quality-of-life as the disease advances (Garre-Olmo et al., 2010, Cheng et al., 2012b). Animal models of AD consistently show learning and memory impairments with synaptic plasticity deficits and some psychiatric-like symptoms (e.g. apathy, anxiety-like, depression-like behaviors) (Nistico et al., 2012, Lecanu and Papadopoulos, 2013, Yan et al., 2013). Biochemical analysis of post-mortem AD brains revealed extensive cholinergic deterioration, hippocampal volume shrinkage, and decreased norepinephrine transporter expression and activity (Perry, 1980, Bobinski et al., 2000, Gulyas et al., 2010). Additionally, non-invasive imaging procedures in AD patients indicate basal forebrain atrophy, changes in local amygdala structure, and hippocampus volume loss (Teipel et al., 2005, Mueller et al., 2010, Cavedo et al., 2011, Grothe et al., 2012). Similar findings regarding amyloid and tau biomarkers have also been reported in both humans and experimental animals (Sabbagh et al., 2013).

Definitive diagnosis of AD requires the presence of both extracellular deposits of amyloid proteins termed senile plaques and intracellular inclusions of hyperphosphorylated tau proteins, which form neurofibrillary tangles. These neuropathological hallmarks constitute the amyloid hypothesis, which currently is a favorite in explaining AD pathology. Despite tremendous efforts in elucidating AD

etiology and searching for effective therapy, the disease still has no cure. FDA-approved pharmacological treatments (cholinesterase inhibitors and NMDA receptor antagonist) are effective in alleviating symptoms in only half of the AD patient population with limited therapeutic window and serious side effects (e.g. nausea, vomiting, liver damage). Thus, there is an urgent need for innovative strategies that can halt or reverse AD progression. Recently, anti-amyloid therapies, dieting, and physical activity have emerged as promising preventative treatments of AD (Morgan, 2011, Erickson et al., 2012, Shah, 2013). Among these, regular exercise has received an enormous recognition for exerting a beneficial effect on the brain function, especially learning and memory.

According to the Center for Disease Control and Prevention, 30 minutes of daily exercise can improve your mood, attention, sleep quality and most importantly, it is the golden key to happiness. Depending on different duration and intensity, exercise can modify learning and memory and related signaling cascades in an inverted U shape curve in which too much or too little exercise is detrimental (Kamijo et al., 2004). Exercise is neuroprotective in various brain insults including acute sleep deprivation (Zagaar et al., 2012), oxidative stress (Vollert et al., 2011), Parkinson's disease (Tuon et al., 2012), chronic cerebral hypoperfusion (Cechetti et al., 2012), and cerebral hypoxia (Gozal et al., 2010). Several other exercise paradigms show an enhanced performance in learning and memory tasks of exercised rats compared to sedentary controls (Falls et al., 2010, Kennard and Woodruff-Pak, 2012). At the cellular level, these behavioral adaptions induced by exercise translate to stronger synaptic connections, sprouting of dendritic spines, neurogenesis, and robust long-term potentiation (LTP), a model of

learning and memory (Stranahan et al., 2009, Hotting and Roder, 2013, Lee et al., 2013). Physical exercise is beneficial to cognitive function, and the combination of both mental and physical activity provides a synergistic beneficial effect on brain health (Curlik and Shors, 2013). In addition, exercise is known to exert an anxiolytic effect in humans even beyond the therapeutic efficacy of anxiety-reducing agents (Wipfli et al., 2008); thus making exercise a potential treatment for clinical anxiety disorders (Asmundson et al., 2013).

Given the opposite effects that AD pathology and exercise training have on cognitive and non-cognitive functions, it will be of interest to investigate the effect of regular exercise in learning and memory impairment associated with AD pathology. Through 16 prospective studies, a meta-analysis has quantified that active people will be less likely to develop Alzheimer's disease or Parkinson's disease (Hamer and Chida, 2009) compared to sedentary people. In this study, we thoroughly investigate the impact of moderate treadmill exercise in a rat model of AD-like pathology using a 3-tiers design that consists of behavioral assessments, electrophysiological recordings, and molecular analysis. Our model of AD was achieved by continuous i.c.v. infusion of amyloidogenic $A\beta_{1-42}$ peptides (250 pmol/day, 2 weeks length). Our exercise protocol is considered to be a moderate regimen of forced running on motorized treadmill. Three hypotheses were tested in our study: 1) moderate treadmill exercise prevents the cognitive (i.e. spatial learning and memory) and non-cognitive (i.e. anxiety-like behaviors) disturbances induced by AD-like pathology; 2) AD-induced suppression of long-term potentiation in Schaffer/collaterals and perforant synapses can be prevented

by moderate treadmill exercise; and 3) Abnormal signaling cascades induced by AD pathology are normalized by prior moderate treadmill exercise.

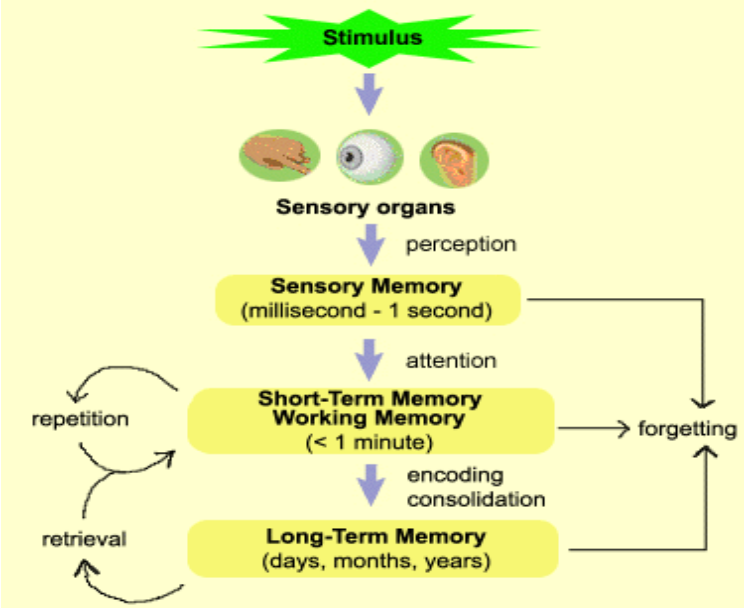
The contribution of this study is significant as successful completion of the study not only may contribute to the discovery of an inexpensive and risk-free treatment of AD but also may reveal molecular targets involved in mediating the beneficial effects of regular exercise on the AD brain. Eventually, preventative treatments of AD may lead to the use of exercise mimetics, which may be effective in delaying the onset of AD and possibly reversing the disease particularly in physically challenged individuals. If indeed regular exercise proves to be neuroprotective in AD, the caregiving burden and spiraling financial costs of caring for AD patients will be greatly reduced.

2. LITERATURE REVIEW

2.1. Learning and memory

Learning and memory are closely related cognitive processes through which information about the surrounding environment is recognized (Bailey et al., 1996). Learning is defined as a process that leads to modifications of a subsequent behavior, whereas memory is the ability to remember past events; or simply just a record left by a learning process (Okano et al., 2000). Based on the neural circuits that get activated and the duration, memory can be divided into different types including sensory, short-term and long-term memory.

Sensory memory, lasting about miliseconds to 1 second, results from initial perception of the external environment by touch, smell, and/or hearing. This type of memory allows the brain to weigh the importance of the incoming information for further processing. Once sensory memory is attentive enough, it will be converted to short-term memory or working memory (e.g. reading a sentence). Short-term memory allows the temporary storage of information for immediate action at the moment, lasting less than 1 minute; it is powerful but has a very limited capacity. Thus, short-term memory can be encoded to long-term memory, lasting days, months and even life-time, or can be simply forgotten (Figure 1, upper panel).



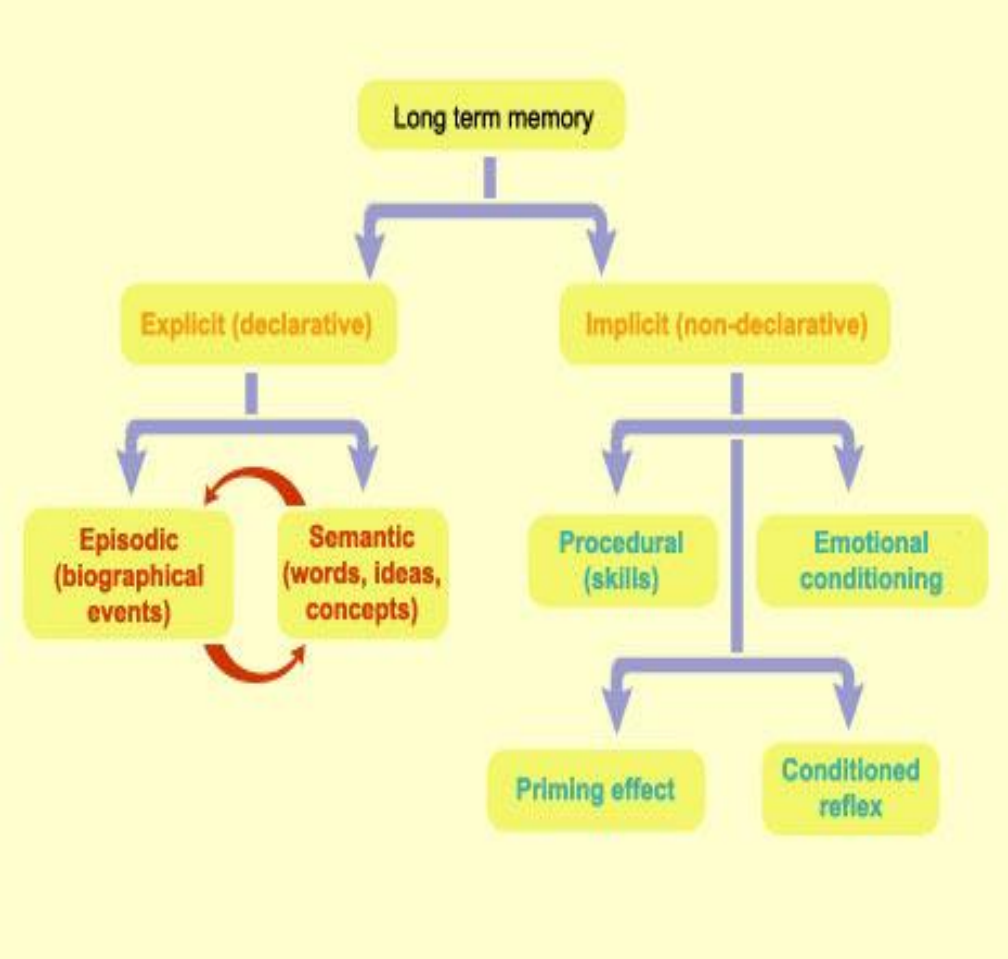


Figure 1. *Upper panel:* different types of memory*, Lower panel:* types of long-term memory (Taken from http://thebrain.mcgill.ca/flash/d/d_07/d_07_p/d_07_p_tra/d_07_p_tra.html)

Long-term memory is further categorized into declarative or non-declarative mamory. Non-declarative, or implicit memory is the type of memory that can be retrieved without conscious attempt (e.g. driving a car). It is more of a procedural skill or emotional conditioning memory (Figure 1, lower panel) (Squire et al., 1988). The brain areas involved in this type of memory are the neostriatum, the caudate nucleus and putamen (Saint-Cyr et al., 1988, Setlow and McGaugh, 1999, Maddox and Ashby, 2004). Declarative, also called explicit memory, requires conscious recall of all past events, which depends heavily on the integrity of the medial temporal lobe, especially the hippocampus and the diencephalon (Malamut et al., 1992, Setlow and McGaugh, 1999, Grunwald et al., 2003, Pergola et al., 2012).

2.2. The hippocampus

The hippocampus is a bilateral, laminar structure that lies in the medial temporal lobe of the brain. It is a critical region implicated in learning and memory, especially spatial memory (Olton et al., 1978). The hippocampus named after the Latin word "seahorse" and consists of two interlocking "Cs" sub-regions as demonstrated in figure 2. The first "C" refers to the pyramidal cells of the Cornu Amonis (CA1 - CA3 areas), while the second "C" is the granule cells of the dentate gyrus (DG) (Amaral and Witter, 1989). Even though the literature has generally and extensively demonstrated the hippocampus as a whole is where memory starts and temporarily stored, studies have reported different functions of each separate sub-region. For example, the dentate gyrus acts as filter of new incomming information or pattern separation; thus making it an effective gateway into the CA3 storage, an area that encodes new spatial information

(Kesner, 2013b, a, Vivar and van Praag, 2013). Additionally, alterations along the transverse axis of the hippocampus result in various memory impairments. The septal (dorsal) area of the hippocampus is more involved in spatial learning and memory while its temporal (ventral) part affects motivational and emotional learning (Moser and Moser, 1998, Potvin et al., 2006, Bast, 2007).

One distintive feature of the hippocampus is its unidirectional trisynaptic connection (Figure 2). Incoming information enters the hippocampal formation via the entorhinal cortex, whose nerve terminals synapse onto the granule cells of the DG via the perforant pathway. Next, information continues to travel along axons of the DG via the mossy fiber pathway and synapses onto the CA3 area pyramidal cells. Then, the last connection namely the Schaffer collaterals pathway, connects the CA3 area to the CA1 area pyramidal cells. Cellular analogues of learning and memory (i.e. long-term potentiation) can be modeled by applying repetitive high frequency stimulation to one of those pathways.

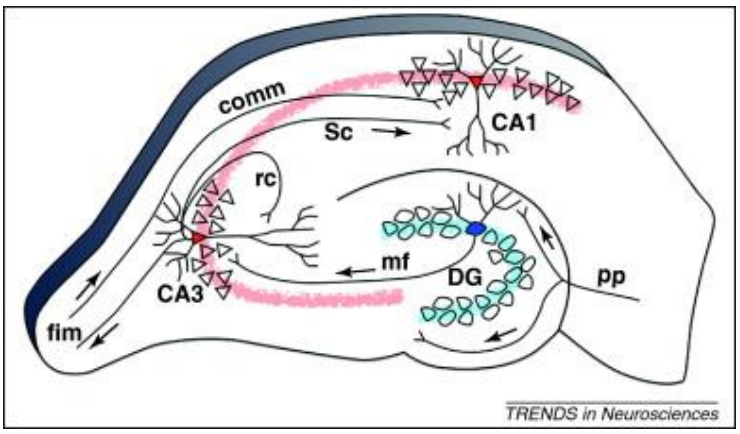


Figure 2. The unidirectional trisynaptic circuit of the hippocampal formation. Fibers project from the entorhinal cortex to the dentate gyrus via the perforant pathway (pp). Axons of the dentate gyrus then synapse onto the pyramidal cells of the CA3 area via the mossy fiber pathway (mf). Then, CA3 area connects to the CA1 area via the Schaffer collateral pathway (Sc). Also, the commissural pathway (comm) sends projections to the contralateral hippocampus. Taken from (Yassa and Stark, 2011).

2.3. The hippocampus and memory

The hippocampus serves as a temporary reservoir of new information before being sent to the cerebral cortex for permanent storage (Sutherland et al., 1989). Much of the current understanding about the role of the hippocampus in learning and memory processes owes a great debt to the famous case of patient Henry Molaison (H.M). The catastrophic bilateral surgical removal of H.M.'s medial temporal lobe structure to control his life-threatening seizures forever left him in the same moment. The surgery had been a precious gift to neuroscience, especially learning and memory research. After the surgery, H.M. experienced anterograde amnesia, a condition in which the patient is not capable of forming new memories (Scoville and Milner, 1957). Since then, the hippocampus function has been extensively studied in humans, non-human primates, and rodents. It is well recognized that lesions or damage to the hippocampus impaired spatial learning and memory (Faraji et al., 2008, Brady et al., 2010, Gomez et al., 2012).

2.4. Animal tests for learning and memory

Learning and memory is an abstract concept that has been studied extensively using behavioral paradigms and electrophysiological experiments in animals. Behavioral testings allow us to "score" learning and memory in rodents while electrophysiological studies reveal synaptic events during the process of learning and memory.

2.4.1. The rodent mazes

In rodent, spatial learning and memory is strictly hippocampus dependent and the maze is a common test for this type of memory (short-term and long-term) (Wenk,

2004). Even though there are various type of mazes, all share the same principle that uses visuospatial cues, the animals must learn the location where rewards (e.g. food or safety) can be found.

2.4.1.1. The radial arm maze (RAM)

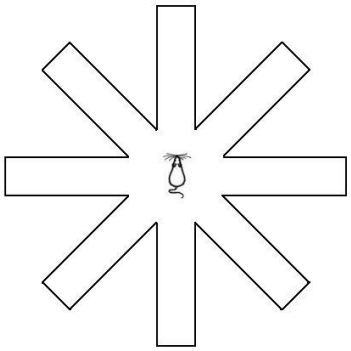


Figure 3. The radial arm maze.

The radial arm maze is an effective assay of spatial learning and memory (short-term and long-term) using food rewards. The first RAM, developed by Olton and Samuelson in 1976, consists of eight horizontal arms radiating from an opened central area (Figure 3) (Olton et al., 1978). For each learning trial per day, the food bait is placed at the end of four out of the eight arms and remains constant throughout the training period. After food deprivation, each animal is placed in the central area and allowed to explore the arms. Each training trial lasts about 5 minutes or whenever all food is consumed. During a particular learning trial, the control animal should avoid entering the baited arm more than once after finishing the food pellets. Thus, short-term memory (working memory) is quantified by the number of times that the animal enters the baited arm more than once (errors). Similarly, long-term memory (reference memory) can be inferred from errors of entering an unbaited arm. The RAM is effective

in the sense that it allows simultaneous assessments of both short-term and long-term memory. However, food odors and deprivation and/or smells from other animals may be possible disadvantages of the RAM.

2.4.1.2. The Morris water maze (MWM)

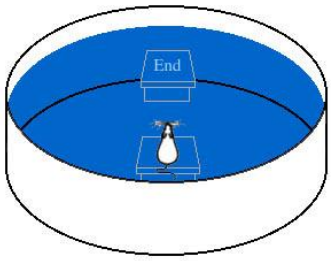


Figure 4. The Morris water maze.

First invented by and named after Richard Morris in 1981, the MWM still remains to be the most widely used maze in behavioral neuroscience that focuses on spatial learning and memory. Typically, rodents are placed in a circular pool filled with opaque water and the only escape route is to locate a hidden platform at a fixed location given visual cues on the surrounding walls (Figure 4) (Morris et al., 1982). The concept behind the MWM is that in a certain spatial environment, place cells in the hippocampus form a unique pattern field on the cognitive map (O'Keefe, 1976). Basically, animals are allowed learning trials in which spatial memory can be inferred from swimming paths toward the platform and escape latency. Normally, control animals will quickly learn the location of the hidden platform with reduced escape latencies and shorter and more direct swimming path when the subsequent trials continue. The animal is also given a post-training probe test trial (lasting 60 seconds) in which the platform will be removed. The well-trained animal with intact spatial memory

would swim toward and begin searching at the location where the platform had been previously located. This spatial bias can be used as an index of spatial learning and memory. The MWN eliminates confounding factors such as food odor and deprivation associated with the RAM. However, the MWM is not quite ideal in hippocampal lesions studies as overtraining (large number of trials in many days) can make these animals perform well in the probe test (Morris et al., 1990). Additionally, it is possible that rodents just passively swim/float until they bump into a subject without using the visuospatial cues.

2.4.1.3. The radial arm water maze (RAWM)

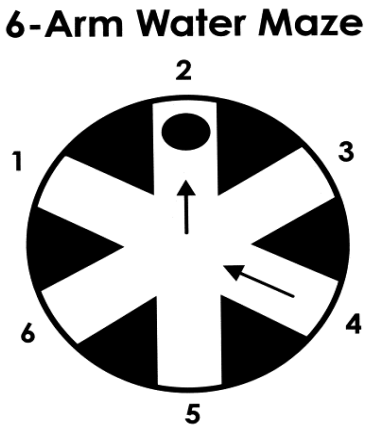


Figure 5. The radial arm water maze.

The RAWM is a hybrid of the MWM and RAM. First developed by Buresova and colleagues in 1985, the RAWM proves to be a valuable tool in assessing spatial learning and memory performance in rodents. The RAWM is a black circular pool with six swim arms leading radially from an opened center area (Figure 5) (Buresova et al., 1985). In this paradigm, the animals are forced to navigate through the arms in order to reach the hidden platform, which is placed at the end of a goal arm. The goal arm is randomly

assigned to each animal. The paradigm involves learning trials, short-term and long-term memory tests. The advantages of both the RAM and MWM are fully attained in the RAWM procedures. Thus, in this study we used RAWM to simultaneously evaluate spatial learning, working and reference memory while the disadvantages of food odor and deprivation are completely avoided. Our experiment protocol is further detailed in the Materials and Methods section.

2.4.2. Electrophysiological investigations

The hippocampus, structured into well-defined homogenous cell layers, has been extensively investigated at the synaptic level *in vitro* (in hippocampal slices) and *in vivo* (in anesthetized animals). Experiments with hippocampal slices provide feasibility for pharmacological manipulations (i.e. drug administration) and correct positioning of the electrodes in the sub-regions. However, recordings from slices do not necessarily reflect the physiologic conditions due to circuitry disruptions, neurotransmitter alterations, altered phosphorylation of protein, and temperature variations (Ho et al., 2004).

We performed extracellular electrophysiological recordings in both the CA1 and DG sub-regions of the hippocampus in urethane-anesthetized animals. Urethane is proven to be the most suitable anesthetic agent for *in vivo* recordings (Maggi and Meli, 1986). Proper positioning of the stimulating and recording electrodes into any of the hippocampus synaptic pathways can produce a postsynaptic response called population spike (pspike) (shown in figure 6) that can be analyzed into two parameters: 1) field excitatory post-synaptic potential (fEPSP) slope and 2) pspike amplitude. The fEPSP

slope represents synaptic strength while pspike amplitude indicates the number of neurons that reached threshold and fired. Repetitive stimulation of any hippocampal pathway via high frequency stimulation (HFS) results in long-lasting changes of the synapses of that particular pathway (synaptic plasticity). Depending on the stimulation protocol, the changes could be an increase (long-term potentiation) or decrease (long-term depression).

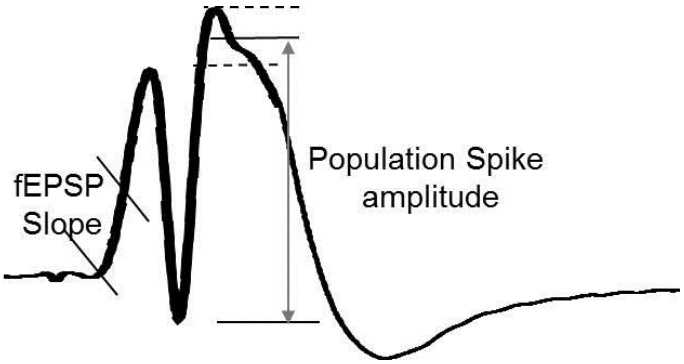


Figure 6. A trace of postsynaptic response in the hippocampus.

2.5. Hippocampal long-term potentiation (LTP)

Synaptic plasticity in the form of enhanced synaptic strength is called long-term potentiation (LTP). Based on the fact that during learning and memory process, synaptic transmissions within and between neurons are enhances, LTP is considered to be the closest cellular model of learning and memory (Bliss and Collingridge, 1993). First discovered in 1966 by Bliss and Lomo in rabbit hippocampus, LTP has given scientists valuable knowledge regarding the molecular pathways involved in learning and memory (Lomo, 2003). Since its discovery, many forms of synaptic plasticity (short vs. long) have been described and are thought to be mediated by a multitude of pre-synaptic and post-synaptic mechanisms (Nayak and Browning, 1999, Nicoll, 2003, Lu and Hawkins, 2006).

LTP is observed in all sub-regions of the hippocampus but the molecular mechanisms behind LTP of each sub-region are varied. For instance, LTP of the mossy fiber pathway is totally independent of postsynaptic calcium (Mellor and Nicoll 2001), thus making this type of LTP independent of N-methyl-D-aspartate (NMDA) receptors (Bortolotto et al., 2005). On the other hand, LTP of the perforant and Schaffer collaterals synapses can only be evoked when there is activation of NMDA receptors and subsequent postsynaptic calcium entry (Lynch et al., 1990, Regehr and Tank, 1990). In our study, we induced LTP in the CA1 and DG areas using train(s) of HFS. A single train of HFS produces only an early phase of long-term potentiation (E-LTP) while multiple trains of HFS induce a longer plasticity called late phase LTP (L-LTP).

2.5.1. Early phase LTP (E-LTP)

E-LTP, a cellular analogue of short-term memory, is a transient phenomenon of increased synaptic strength that lasts up to 3 hours. This type of synaptic plasticity is independent of protein synthesis and requires the activation (phosphorylation) of Ca^{2+}/calmodulin dependent protein kinase II (CaMKII), which in turn increases the turnover and conductivity of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Briefly, single train of HFS depolarizes the pre-synaptic membrane causing subsequent glutamate release. The released glutamate activates post-synaptic glutamate receptors. The post-synaptic membrane is then sufficiently depolarized and the Mg^{2+} blockage of NMDA receptors is removed allowing the entry of Ca^{2+} into the cell. Ca^{2+} influx dissociates calmodulin from neurogranin and activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which autophosphorylates once

becoming activated. CaMKII activation can also enhance AMPA receptor trafficking to the membrane. Calcineurin (PP2B) is a phosphatase that can shut off the constitutive activation of CaMKII, returning the synapses to its normal state (Figure 7A).

2.5.2. Late phase LTP (L-LTP)

A more durable form of LTP (L-LTP), lasting more than 3 hrs and up to days, can be evoked by application of multiple trains of HFS to the desired pathway. L-LTP is dependent on *de novo* protein synthesis, which requires activation of kinases and transcription factors. Unlike E-LTP, a more focused Ca^{2+} entry into the postsynaptic membrane leads to phosphorylation of cAMP response element binding protein (CREB) via Ca^{2+}/ calmodulin dependent protein kinase IV (CaMKIV) and the mitogen-activated protein kinase (MAPK-ERK1/2) pathways, thus initiating transcription of target genes implicated in synaptogenesis, neuronal survival, and learning and memory (Figure 7B).

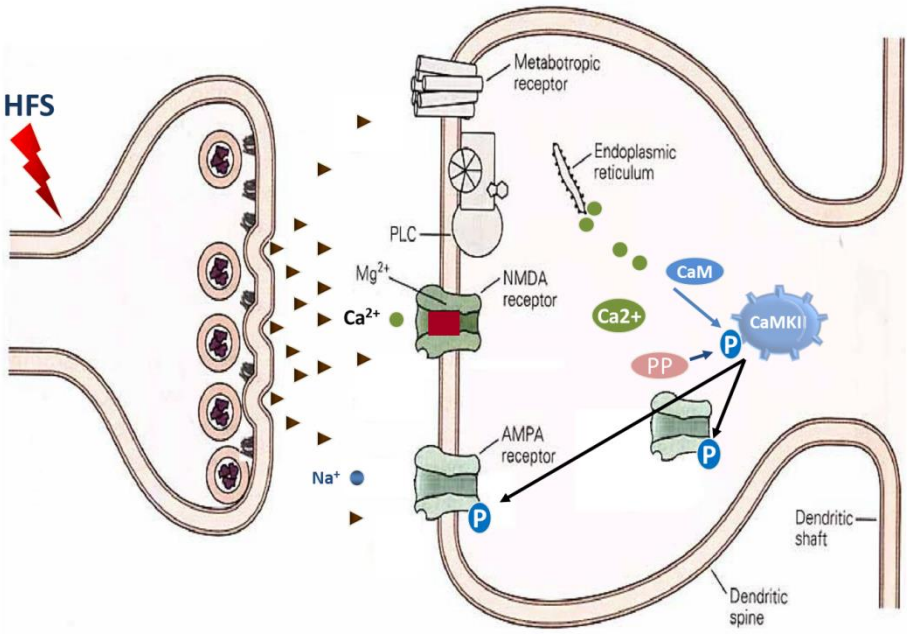


Figure 7A. Schematic diagram of E-LTP molecular expression. Single HFS produces a rise in postsynaptic Ca^{2+}, which can phosphorylate (P) AMPAR receptors to enhance their conductance capability and insertion onto the postsynaptic membrane. Once activated, CaMKII remain to be active via autophosphorylation even though Ca^{2+} amount may return back to the basal level and only phosphatase (PP) can halt this constitutive activity. Adapted from (Kandel and Schwartz, 2001).

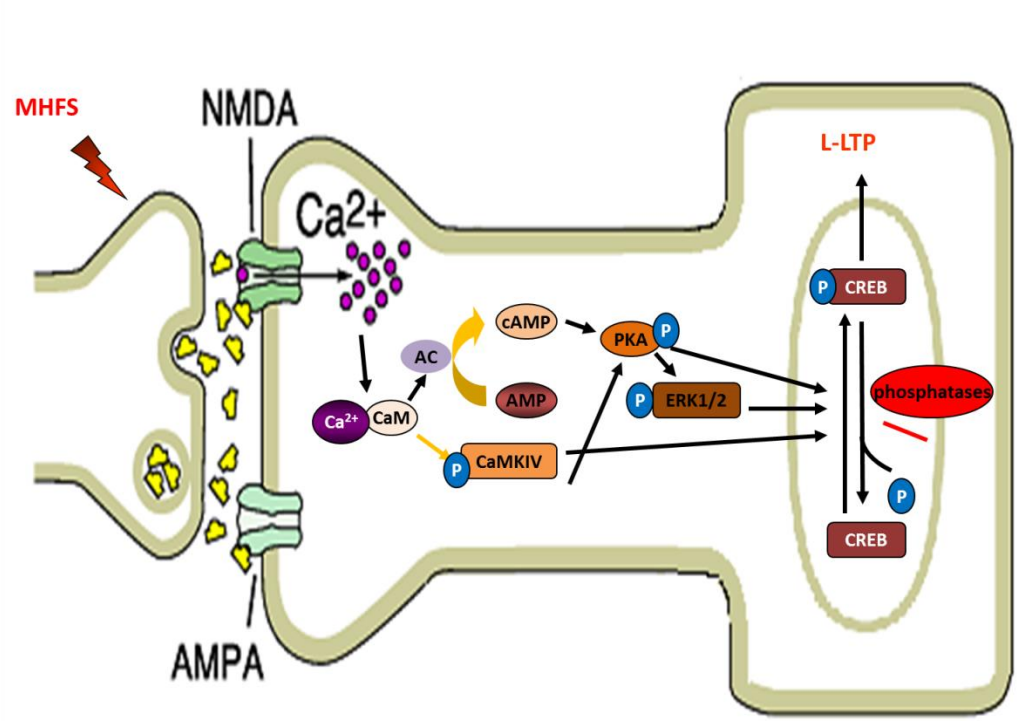


Figure 7B. Induction of L-LTP via multiple trains of HFS. Compared to E-LTP induction, a more focus Ca^{2+} influx activate the CaMKIV and PKA-MAPK (ERK1/2) pathways, that can subsequently phosphorylate CREB and turn on CRE-mediated transcriptions, which are necessary for synapse formation, neuronal survival, and memory formation Adapted from (Kandel and Schwartz, 2001).

2.6. Signaling molecules involved in learning and memory, and LTP:

Various signaling molecules have been shown to play a pivotal role during learning and memory processes as well as the LTP phenomenon. The following section provides a brief review of these molecules in term of structure, memory and LTP-related functions.

2.6.1. N-methyl-d-aspartate (NMDA) receptors:

The NMDA receptor, which belongs to the ionotropic glutamate receptor family, is attached to a calcium channel that is usually voltage-dependently blocked by Mg^{2+} under resting condition (Figure 8). The NMDA receptor is a hetero-tetramer complex that consists of two NR1 and two NR2 subunits. Glutamate binds to the site on NR2 while the co-agonist glycine is the ligand on the NR1 subunit. Functional studies reveal that NMDA glutamate receptors play a critical role in spatial memory and LTP induction, particularly LTP of the perforant and Schaffer collaterals pathways. The selective NMDA receptor antagonist (AP5) directly interferes with spatial and emotional learning and memory and completely blocks LTP (Davis et al., 1992, Elvander-Tottie et al., 2006, Morris et al., 2013). However, exogenous administration of AP5 after a learning task has no effect on retention of prior acquired spatial information nor does it influence the expression of LTP (Morris, 1989). Genetic studies also prove the importance of NMDA receptors in learning and memory. For instance, CA1 area NR1 or NR2A subunits knockout mice exhibited both spatial learning and memory and LTP deficits (Berberich et al., 2007, Place et al., 2012). Additionally, non-spatial learning and memory is also affected in mice without CA1 NMDA receptors (Rondi-Reig et al., 2001). Interestingly,

one related study showed that mice lacking the NR1 subunit in the DG area displayed impaired spatial working memory but intact spatial reference memory (Niewoehner et al., 2007). Together, these results indicate a correlation between memory and LTP and how alterations in NMDA signaling could affect these parameters.

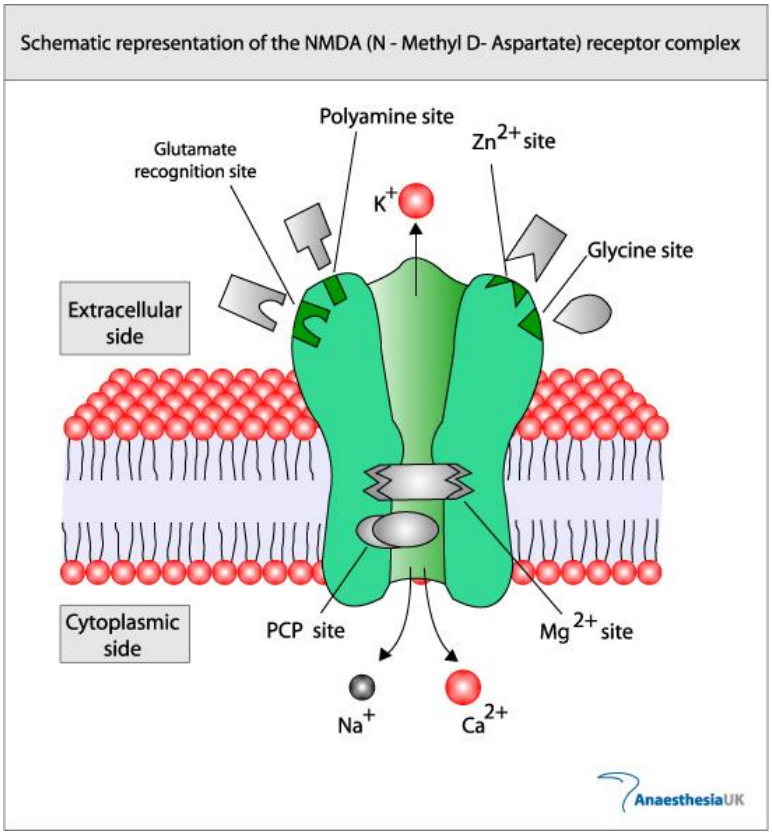


Figure 8. Structure of a NMDA receptor complex. NMDA receptor possesses several ligand binding sites such as glutamate, glycine, polyamine and Zn^{2+}. Under resting condition, the receptor is blocked by Mg^{2+}, which can be removed by changes in membrane voltage. Taken from http://www.frca.co.uk/article.aspx?articleid=100515.

2.6.2. Ca^{2+}/calmodulin dependent protein kinase II (CaMKII)

Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) is a key kinase in the learning and memory process. Autophosphorylation by exposure to the Ca^{2+}/calmodulin complex turns this kinase constitutively active despite depleted postsynaptic Ca^{2+}. CaMKII is a dodecamer with the core structure of a catalytic domain and an

autoinhibitory domain (Figure 9) (Hunter and Schulman, 2005). It is the most abundant enzyme in the brain with the predominant isoform, CaMKIIα (Yamasaki et al., 2008). Reduced levels of mRNA and protein expression of CaMKIIα correlate with an impaired spatial learning and memory in a model of epilepsy (Wang et al., 2008a). Furthermore, pharmacological inhibition of CaMKII by KN-93 blocks short-term plasticity and aggravates the suppression of synaptic transmission regardless of high frequency stimulation (Mukhamedyarov et al., 2010).

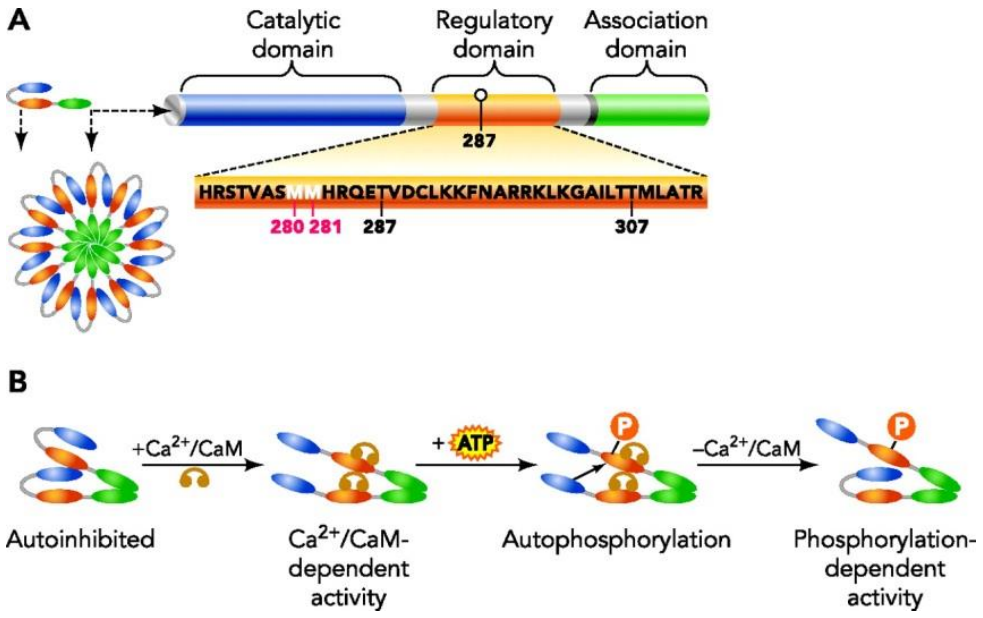


Figure 9. A) A CaMKII monomer consists of catalytic, regulatory, and association domain, which can assembly into the dodecamer structure, or CaMKII holoenzyme. B) Activation CaMKII. Ca^{2+} enters the postsynaptic cell and binds to calmodulin. This complex binds to CaMKII regulatory domain and releases the catalytic domain from the inhibitory effect of the regulatory domain and thus expose Thr286 and result in subsequent phosphorylation of this amino residue. Once active, CaMKII remains to be autophoshorylated. Taken from (Couchonnal and Anderson, 2008).

2.6.3. Calcineurin

In the hippocampus, phosphatases including calcineurin (PP2B) are responsible for deactivating kinases-induced phosphorylated substrates. In particular, PP2B can

return CaMKII constitutive activity to basal level via dephosphorylation. Activation of calcineurin via the calcium sensing calmodulin complex removes its autoinhibitory domain and results in phosphatase activation (Figure 10) (Rumi-Masante et al., 2012). It has been shown that synaptic plasticity in the hippocampus involves bidirectional control of kinase/phosphatase switch (Belmeguenai and Hansel, 2005). For example, previous studies from our lab indicate upregulation of basal levels of PP2B during stress and AD pathology which possibly explain an impaired LTP, enhanced long-term depression (LTD), and memory deficits in those rats (Gerges et al., 2004, Srivareerat et al., 2009).

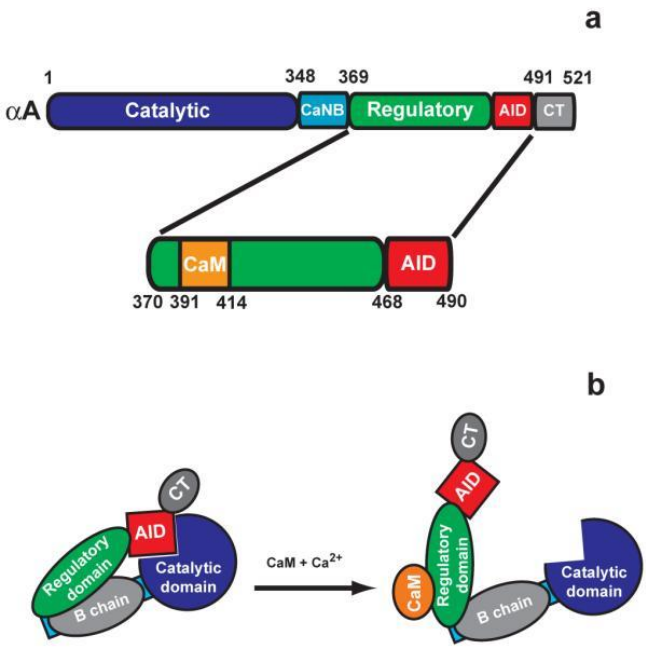


Figure 10. a) Structure of calcineurin (PP2B) includes a catalytic, regulatory and autoinhibitory domain (AID). The regulatory domain of PP2B contains the calcium sensor calmodulin (CaM) binding site. b) Activation of PP2B. Conformational change, results from binding of CaM to the regulatory domain, displaces the AID and thus activates PP2B. Taken from (Rumi-Masante et al., 2012).

2.6.4. cAMP response element binding protein (CREB)

cAMP response element binding protein (CREB) is a transcription factor that plays an important role in memory formation and LTP (Pittenger and Kandel, 1998, Alberini, 2009, Kida, 2012). CREB is activated via phosphorylation at Serine 133 by several kinases (Shaywitz and Greenberg, 1999). Upon depolarization during learning and memory or tetanic stimulation, influxed Ca^{2+} forms a complex with calmodulin leading to cAMP dependent protein kinase IV (CaMKIV) activation, which eventually phosphorylates CREB and turns on CREB transcriptional activity (Figure 11). Mice expressing genes with increased level and activity of CREB show an enhanced memory and lower-threshold LTP (Du et al., 2000, Barco et al., 2005). In contrast, CREB inhibition or suppression of CREB phosphorylation via genetic approach or pharmacologic agent reduces LTP magnitude and increases LTD expression (Fan et al., 2010, Middei et al., 2013), and even causing neuronal death (Jancic et al., 2009).

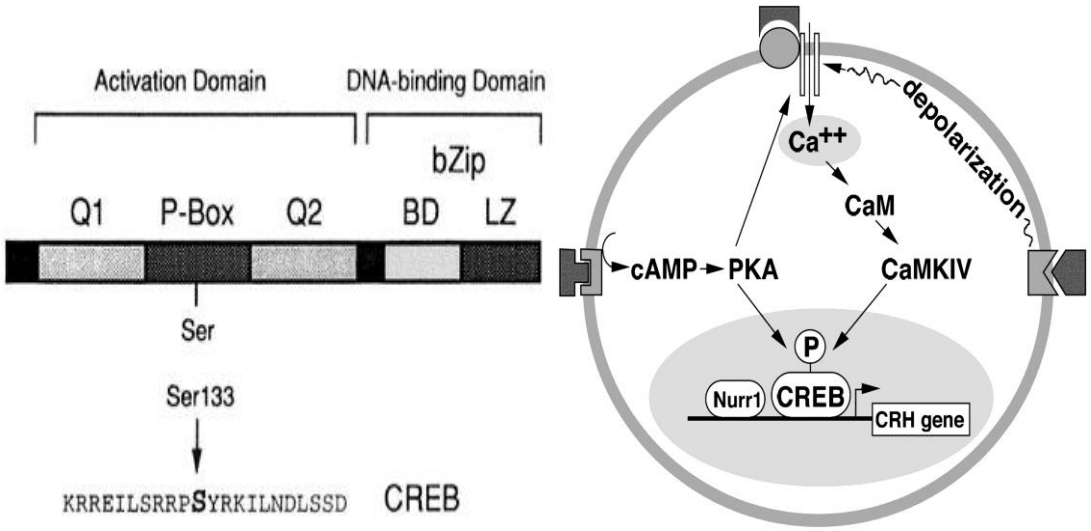


Figure 11. *Left panel*: structure of CREB consists of an activation domain, which contains the glutamine-rich regions Q1 and Q2 and a DNA-binding domain (bZip regions BD and LZ) in addition to the P-Box. *Right panel*: CREB-mediated transcription. The Ca^{2+}/calmodulin (CaM) and cAMP signaling pathways activate protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) respectively, which in turn phosphorylate CREB at Ser133 and result in CREB-mediated transcriptions. Taken from (Fimia et al., 2001, Yamamori et al., 2004).

2.6.5. Ca^{2+}/calmodulin dependent protein kinase IV (CaMKIV)

Similar in structure to that of CaMKII, CaMKIV possess a Ca^{2+}/calmodulin (CaM) site, once occupied, the complex will turn on the basal kinase activity. Further phosphorylation of the CaM-bound CaMKIV at a threonine residue on its activation loop will keep CaMKIV activity on an autonomous mode, which is necessary for transcriptions (Chow et al., 2005). Multiple trains of HFS activate the calcium signaling pathway upon which CaMKIV phosphorylates CREB (Figure 11). Targeted deletion of CaMKIV in mice impairs L- LTP, long-term memory and CREB phosphorylation but leaves E-LTP and short-term memory intact (Ho et al., 2000, Kang et al., 2001, Lee et al., 2009). In contrast, overexpression of CaMKIV not only enhances LTP and spatial learning and memory but also rescues memory deficit associated with aging (Fukushima et al., 2008).

2.6.6. Extracellular signal-regulated kinase 1/2 (ERK1/2)

Long-term memory consolidation requires the activation of several kinases including ERK1/2 in addition to *de novo* gene expression and protein synthesis (Giovannini, 2006, Xia and Storm, 2012). ERK general structure consists of the catalytic loop domain and hydrophobic skeleton (brown color) (Figure 12). Phosphorylation of two residues in the activation segment converts ERK1/2 from inactive to active state (Roskoski, 2012). ERK1/2 has several substrate targets including transcription factors, which results in promoting eventual transcription process (Mao et al., 2004, Tiraboschi et al., 2004). Also, ERK1/2 activation plays a pivotal role in the associative and contextual memory consolidation (Kamei et al., 2006, Huang et al., 2010).

Various signals as such BDNF, G-protein coupled receptors, repetitive stimulation, and tyrosine kinases can activate the MAPK/ERK pathway. This cascade is shown to involve in hippocampal LTP induction. For example, selective inhibition of MAPK blocks its activation in addition to a marked attenuation of LTP induction but does not affect the expression of established LTP (English and Sweatt, 1997). In addition, other studies report that LTP-induced phosphorylation of MAPK can directly modulate CREB signaling and its related transcription processes (Lonze and Ginty, 2002).

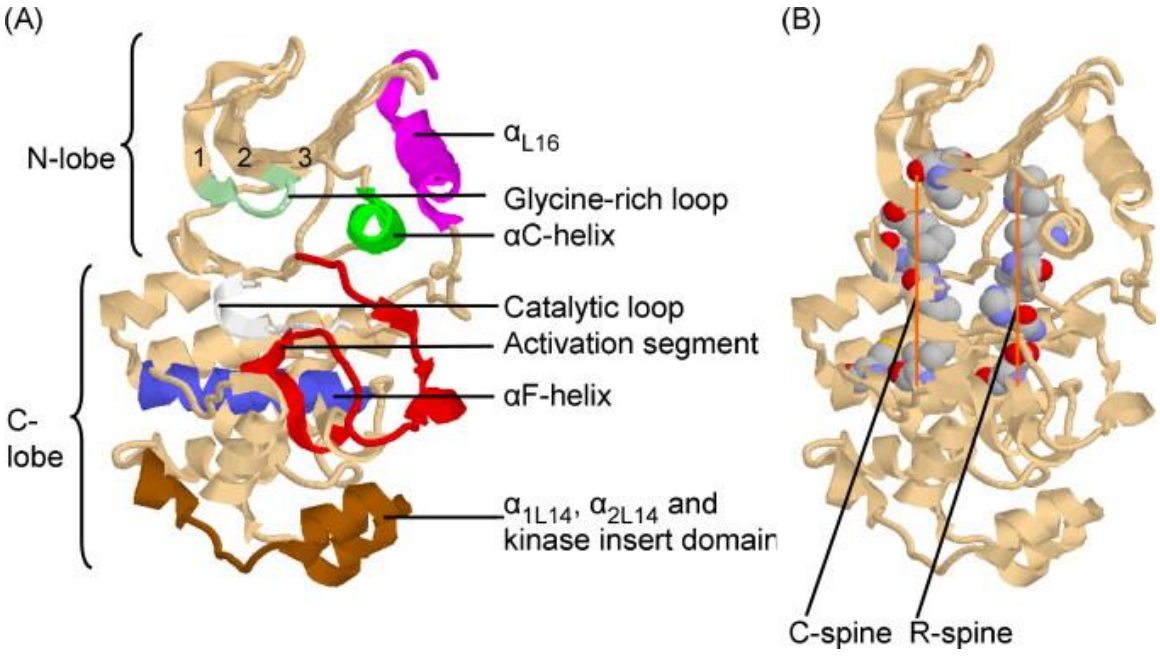


Figure 12. A) Ribbon structure of human ERK2, B) The catalytic and regulatory regions are shown by the orange lines. Taken from (Roskoski, 2012).

2.6.7. Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is an important molecule in the CNS functions, especially in learning and memory processes (Leal et al., 2013). BDNF can be synthesized in neuronal tissues and then sorted via the regulated secretory and constitutive secretory pathways (Figure 13) (Lu et al., 2005). BDNF is a pivotal neurotrophic factor that is critical for neurogenesis, cognition, and plasticity (Rossi et al., 2006, Tyler et al., 2006). BDNF acts as a potent mediator for cell proliferation, differentiation, survival, and maturation in the DG (Wu et al., 2008). BDNF has a neuroprotective effect in learning and memory impairment (Griesbach et al., 2009). Brain insults, LTP induction, and spatial learning all increase the expression of hippocampal BDNF mRNA (Castren et al., 1993a, Durany et al., 2000, Silhol et al., 2007).

On the other hand, BDNF ablation or tropomyosin receptor kinase B (TrkB) truncation impairs spatial memory (Saarelainen et al., 2000, Adlard et al., 2010), and suppresses LTP induction (Zhou et al., 2000, Hennigan et al., 2009); but surprisingly, reducing TrkB expression can actually decrease lesion size in the presence of ischemic stroke (Kraemer et al., 2005). However, the fact that these cognitive deficits can be restored by exogenous administration of BDNF proves that BDNF plays an integral role in synaptic plasticity and memory (Shaw et al., 2003, Rex et al., 2006, Kline et al., 2010). Additionally, evidence indicates that exercise-induced BDNF is a key mediator between energy homeostasis and cognitive function (Gomez-Pinilla et al., 2008).

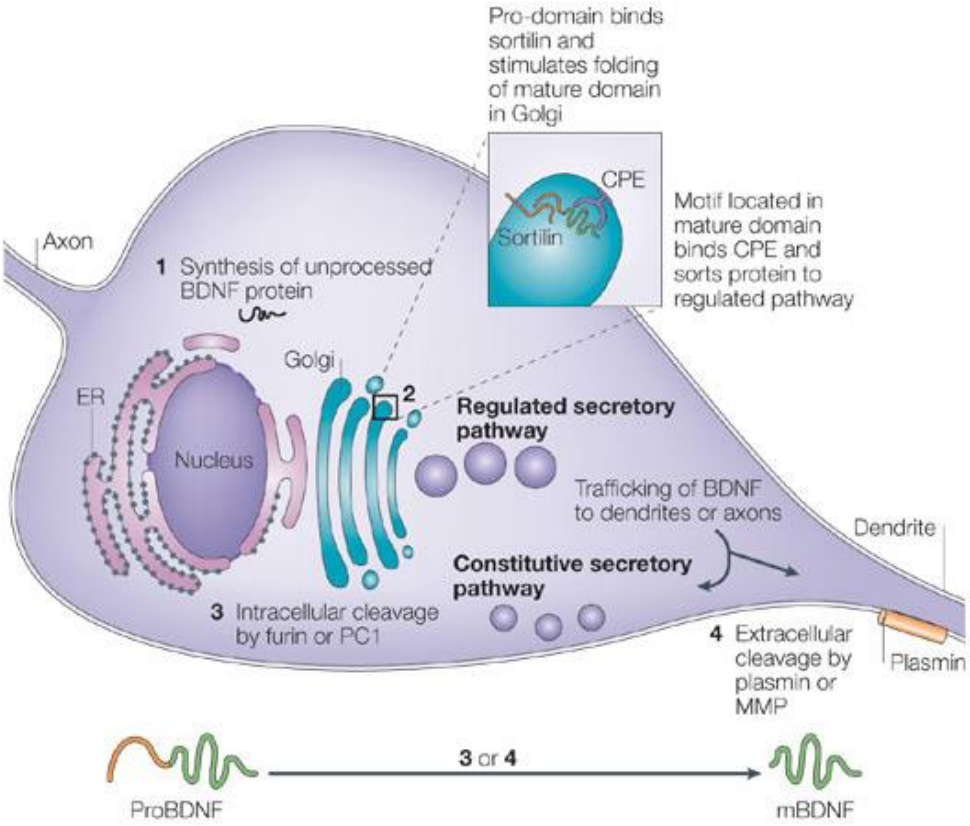


Figure 13. The synthesis and sorting of BDNF in neuronal tissues. (1) BDNF is synthesized from the endoplasmic reticulum (ER) (proBDNF) and then binds to intracellular sortilin of the Golgi in order to stimulate proper folding of the mature domain (2). Binding of a sequence in the mature domain to carboxypeptidase E (CPE) sort BDNF into the large vesicles that contribute to the regulated secretory pathway. In absence of this sequence, BDNF is subjected to the constitutive secretory pathway. After being sorted by either pathway, BDNF are transported to dendrites or axons. ProBDNF is usually cleaved by furin or protein convertase 1 (PC1). Without this intracellular cleavage, proBDNF can also be released by neurons (3) and then further cleaved by plasmin or metalloproteinases (MMP) to yield mature BDNF (4). Taken from (Lu et al., 2005).

2.7. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss accompanied by disorientation and aberrant behavior. According to the Alzheimer's Association, in America one case of AD develops every 70 seconds and this is projected to reach approximately one every 33 seconds by 2050. The pathology of AD progressively affects several brain regions with the hippocampus being the first and most severely affected. This results in gradual neuronal degeneration, which will eventually lead to impaired synaptic plasticity, marked dementia, and non-cognitive disturbances.

2.7.1. Clinical symptoms of Alzheimer's disease

In humans, there are three stages of AD with overlapping symptoms: 1) mild (2 – 4 years), 2) moderate (2 – 10 years), and 3) severe (1 – 3+ years). Clinical phenotypes of AD patients include progressive memory loss as well as non-cognitive disturbances (e.g. anxiety, aggression, agitation). As the disease advances, the patients are completely unable to care for themselves in addition to the irreversible amnesia and a full spectrum of other psychiatric disorders. Thus, the American Psychiatric Association requires evidence for impairment in memory and one other cognitive domain such as language, praxis, visual processing or executive function in order to diagnose AD. However, definitive diagnosis of the disease can only be confirmed by the presence of both extracellular senile plaques and intracellular neurofibrillary tangles. Studies reveal drastic changes in the brains of AD patients termed in which amyloid deposits and tangle formations are gradually built up as the disease worsens (Figure 14).

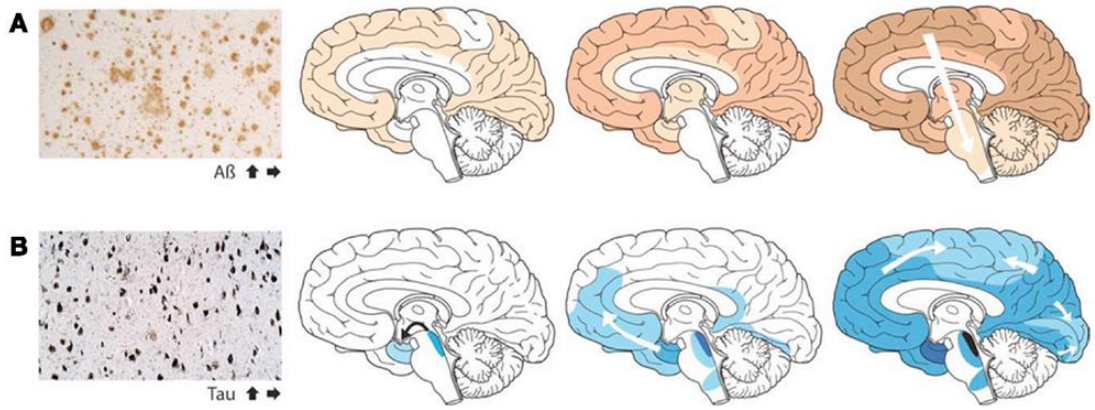


Figure 14. In Alzheimer's disease, the appearance of plaques resulted from accumulation of amyloid proteins (Aβ) and formation of neurofibrillary tangles (NFTs) from hyperphosphorylated tau follow a predictable pattern. (A) Amyloid plaques are first visible in the neocortex and then spreading to the allocortex and then eventually deposit in sub-cortical areas. (B) NFTs begin to form in the locus coeruleus and transentorhinal area, then manifest to the amygdala and interconnected neocortical regions. Taken from (Mattei, 2013).

2.7.2. Pathological hallmarks of Alzheimer's disease

Although the underlying mechanism of AD pathology remains unclear, senile plaques and neurofibrillary tangles have been implicated as the primary neuropathological hallmarks of AD.

2.7.2.1. Amyloid plaques

Amyloid plaques, or senile plaques are extracellular depositions of beta-amyloid (Aβ) proteins that consist primarily of two proteolytic cleavage products of amyloid precursor protein (APP): $A\beta_{40}$ and $A\beta_{42}$ (Durkin et al., 1999, Horsburgh et al., 2000, Gu and Guo, 2013). Soluble Aβ proteins accumulate progressively and finally result in insoluble aggregates (Cirrito et al., 2003) that are extremely toxic to the neurons (Gotz et al., 2008). These plaques are amyloid fibrils with a beta-pleated sheet structure surrounded by neurite dystrophy, activated microglia, and reactive glial cells (Martin-

Rehrmann et al., 2005, Serrano-Pozo et al., 2013). Even though $A\beta_{40}$ is considered to be the most abundant among APP cleavage products, $A\beta_{42}$ is thought to trigger the amyloidogenic cascade that leads to AD (Hardy, 2009). Therefore, some studies have focused on $A\beta_{42}$ radiological-contrasting agents in order to examine ante-mortem brains for early diagnosis and treatment (Perrin et al., 2009).

How do Aβ aggregate is an important question that should be addressed before coming to understand the pathological consequences of Aβ aggregation. Currently, the nucleation polymerization hypothesis is considered to be highly valuable in explaining the process of Aβ fibrillization (Figure 15). According to this hypothesis, soluble monomer Aβ with α-helical structure can be misfolded. Then, the misfolded monomers can dimerize to form the dimers and further oligomerize leading to the formation of micelle-like nuclei with a primary β-structure (the seed). This is the end of the nucleation/lag phase which is slow and thermodynamically unfavorable. Once the seeds form (β-structure oligomers), the reaction enters the elongation/exponential growth phase which is rapid and favorable. The oligomers can assemble into protofibrils structures which are rapidly converted to mature filaments that can be twisted and aggregated into plaques. Therefore, the process of Aβ fibrillization can be characterized by a sigmoidal shape in which the addition of amyloid seeds can reduce the nucleation/lag phase time producing more aggregates with shorter time (red line) (illustrated by Figure 15).

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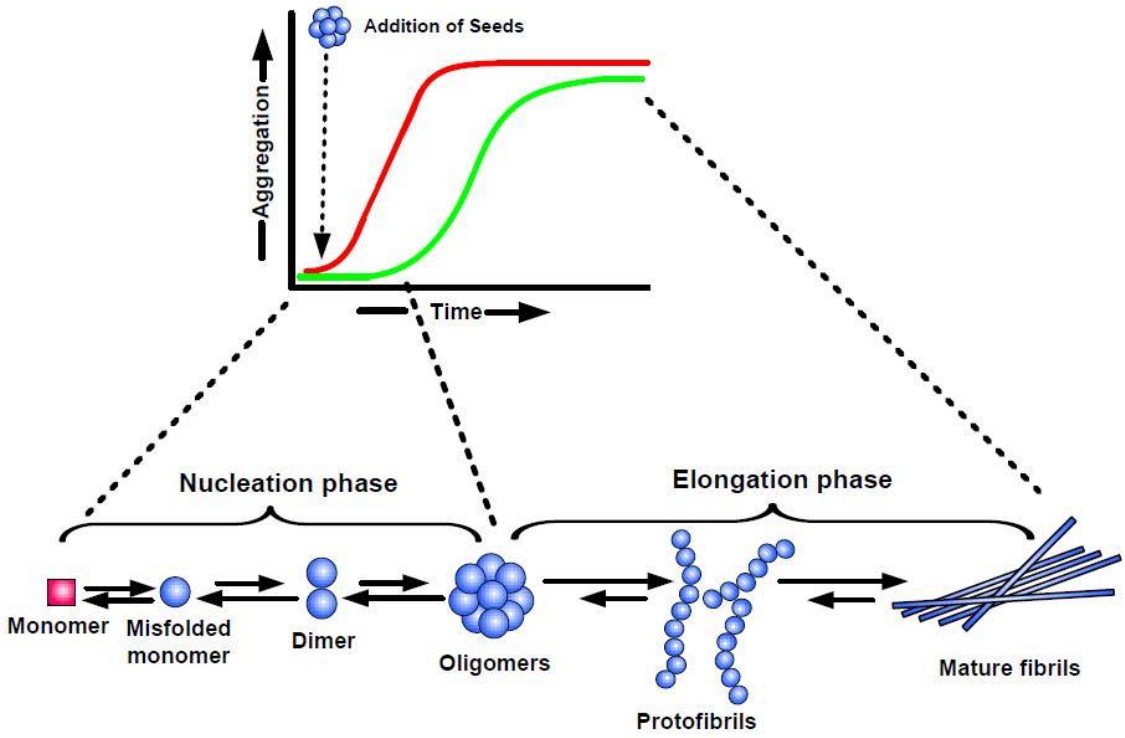

Figure 15. Model of amyloid aggregation consists of 2 phases (i) nucleation phase in which monomers are being converted to oligomers (the nuclei seeds) via conformational changes and (ii) elongation phase in which the nuclei rapidly form fibrils until saturation. Taken from (Kumar and Walter, 2011).

2.7.2.2. Neurofibrillary tangles

Intracellular neurofibrillary tangles (NFTs) are mainly composed of hyperphosphorylated tau, a microtubule-associated protein with a paired helical arrangement (Wisniewski et al., 1976, Santa-Maria et al., 2012). Hyperphosphorylation of tau favors protein aggregation, disrupts microtubule assembly, and impairs axonal transport (Pevalova et al., 2006). Together with amyloid neurotoxicity, tau hyperphosphorylation leads to synaptic failure and neurodegeneration (Terwel et al., 2002). Additionally, Aβ may directly or indirectly interact with tau to promote NFT

production through distinct cellular and molecular pathways (see review by Blurton-Jones and Laferla, 2006).

Similar to amyloid fibrillization, NFTs can be formed *in vivo* from pretangles leading to the formation of hyperphosphorylated tau into paired helical arrangements (fibrils) which eventually form NFTs. However, i*n vitro* tau fibrillization suggests that with anionic inducers (e.g. arachidonic acid, heparin), tau exists in the trapped formation (assembly incompetent) that is in equilibrium with tau fibrillization (Chirita et al., 2003). Through covalent and non-covalent modifications, tau can transform into the unfolded monomers. These unfolded monomers can form intermediates that either progress into the nuclei seeds via an elongation step and finally form fibrils or skip this seeding process and become fibrils. The final fibrils production is equilibrium between the fibril formation and intermediates availability (Figure 16) (Chirita et al., 2003, Kuret et al., 2005). Hyperphosphorylated tau falls off the microtubule assembly and raise intracellular level of tau, which facilitates the interaction between phospho-tau and tau as well as NFTs formation leading to the deficiency in microtubule support. As a result, disassembly of microtubule impairs axonal transport which may lead to the triggering of apoptosis (Gendron and Petrucelli, 2009, Wang et al., 2012).

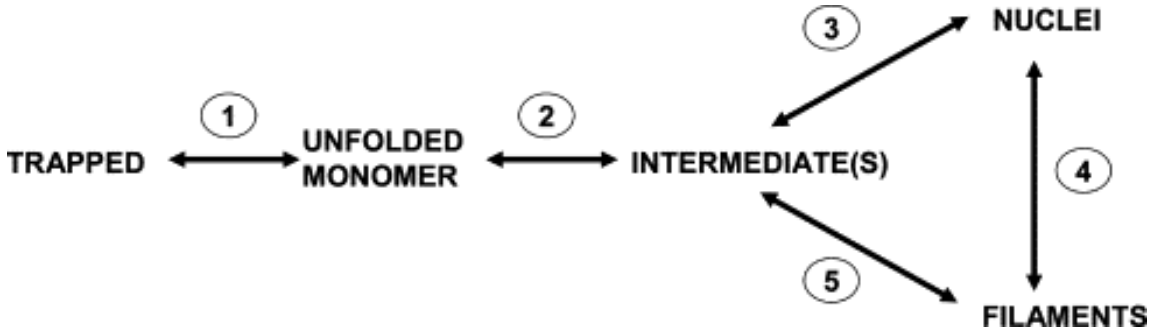

Figure 16. *In vitro* tau fibrillization. Upon anionic inducers, the "trapped" molecules or assembly-incompetent conformations become unfolded monomers (1). These monomers further change to assembly-competent intermediates (2), which can form nuclei (3) or filaments (5). Nuclei can also undergo the elongation process, which is thermodynamically favorable to produce tau filaments (4). Taken from (Kuret et al., 2005).

2.7.3. Amyloid precursor protein (APP) and its processing

Amyloid precursor protein (APP), 695 amino acids in length, is a transmembrane protein, which can be processed to amyloid proteins (primarily Aβ40 and Aβ42) via a family of secretases. Sequential cleavage of APP by the secretases (α, β, and γ) yields numerous Aβ proteins which actively participate in the amyloidogenic and non-amyloidogenic cascades. The non-amyloidogenic pathway begins with APP cleavage by α-secretase into a soluble APP variant (α-APP) and a 83 amino acid C-terminal fragment (C83) retained in the membrane whose further cleavage by γ-secretase yields a small non-pathologic peptide called P3. In the amyloidogenic pathway, β-secretase competes with α-secretase for the APP substrate and produces β-APP and a longer C-terminal fragment (C99) whose subsequent cleavage by γ-secretase results in pathogenic $A\beta_{40}$ or $A\beta_{42}$ (Figure 17). Extracellular amyloid proteins can be taken up by microglia cells whose membranous surface possesses insulin-degrading enzyme (IDE), which can degrade amyloid proteins. Additionally, neprilysin, an endopeptidase, can also degrade Aβ shortly after its production (Mukherjee and Hersh, 2002, Miners et al., 2011). Aβ can

interact with the anionic site of acetylcholine esterase, which facilitates the formation of oligomeric Aβ (Silman and Sussman, 2005, Inestrosa et al., 2008). Furthermore, Aβ can also be taken up via the endocytotic pathway, and along with intracellular production of Aβ from the transGolgi network and endoplasmic reticulum, can be accumulated intracellularly (Echeverria and Cuello, 2002). Thus, it is arguable that intracellular accumulation of Aβ may prime the neurons to be more vulnerable to future attacks of extracellular amyloid plaques and intracellular tangles formation.

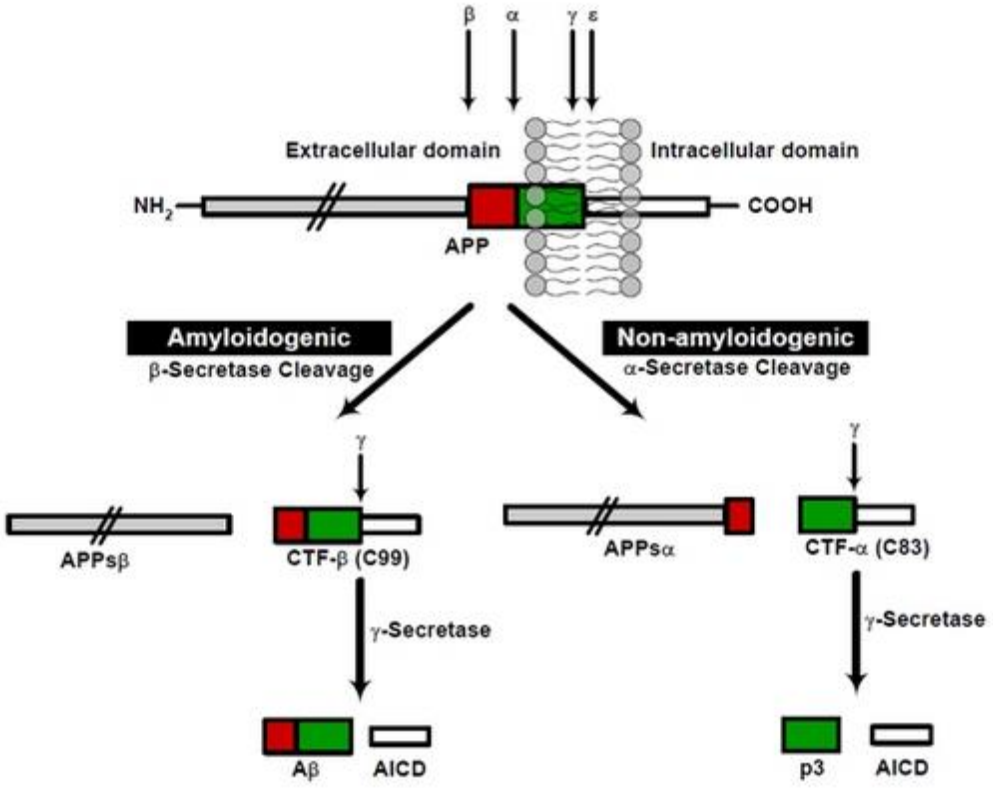

Figure 17. The processing of APP via secretases. APP can be cleaved by either α- or β- secretase and subsequent γ-secretase to yield non-pathogenic P3 fragment or Aβ products respectively. Taken from (Kumar and Walter, 2011).

2.7.4. The amyloid hypothesis:

Current diagnosis of AD i.e. neuroimaging techniques and fluid biomarkers rely heavily on the presence of Aβ accumulation, which is a major basis for the amyloid hypothesis. The amyloid hypothesis simply states that Aβ accumulation is what triggers the pathology and is largely responsible for overall disease progression (Hardy and Allsop, 1991, Hardy, 2009). Although the hypothesis remains inconclusive, imperative genetics seems to support it as familial AD, for instance, is caused by inheritance of mutated genes that code for APP or APP processing proteins such as presenilin 1 (PS1) and presenilin 2 (PS2). Additionally, Down syndrome patients who have three copies of APP gene are more likely to develop familial AD (Schapiro and Rapoport, 1989).

Even though the amount of Aβ accumulation is positively correlated with the degree of cognitive impairment (Naslund et al., 2000, Parvathy et al., 2001, Wilson et al., 2009), soluble Aβ proteins are paradoxically present in the brains of healthy people as well. Additionally, there are cases of AD patients with no presence of amyloid plaques while there is evidence of those toxic amyloid accumulation in brains of non-demented healthy aging individuals (Morris et al., 1996, Shimada et al., 2011). Thus, the amyloid hypothesis fails to account for those special cases. Nevertheless, long-term imbalance between Aβ production and clearance can lead to the formation of insoluble amyloid plaques eventually leading to neuronal cell death and cognitive decline (Cavallucci et al., 2012).

2.7.5. Types of Alzheimer's disease

2.7.5.1. Familial AD

Familial AD, also called as the early onset type of AD, follows an inherited pattern in which the patients experience the disease phenotypes at a very young age (as early as 35 years old) (Filley et al., 2007). Three genes have been identified as causative of familial AD: 1) amyloid precursor protein (APP), 2) presenilin 1 (PS1), and presenilin 2 (PS2) (Tanzi et al., 1996, Borchelt et al., 1997, Price and Sisodia, 1998). Currently, there are more than 230 known mutations in these three genes, which often result in overproduction of toxic amyloid peptides by shifting the APP processing toward the amyloidogenic pathway (Wu et al., 2012).

2.8.5.2. Sporadic AD

Unlike the hereditary early onset of AD, sporadic AD occurs in the elderly population (more than 65 years old) and accounts for the majority of all AD cases. Sporadic AD takes an insidious nature in term of disease pathogenesis but very similar phenotypes compared to the familial type. Even though sporadic AD carries no genetic link component, but having a family member with AD will increase the probability of getting the disease three times. Aging is the most important risk factor of AD. Besides, there are numerous risks factors leading to the pathology including stress, diabetes, high blood pressure, stroke, brain-related injuries, and most importantly carriers of the apolipoprotein E allele (especially the apoE4 variant). Extensive evidence presents various cases of people who have apoE4 copies that markedly increase their risks of getting AD (Coon et al., 2007, Han, 2010, Sadigh-Eteghad et al., 2012). Also, post-

mortem examination of AD brains associated with apoE4 genotype showed reduced cholinergic activity and severe loss of choline acetyltransferase activity (Soininen et al., 1995, Allen et al., 1997). However, having two copies of the apoE4 allele does not necessarily guarantee the chance of developing AD or having no copy of the gene will eliminate the incidence of getting the disease.

2.7.6. Animal models of Alzheimer's disease

Since its first description by the physician Alois Alzheimer (i.e. extracellular deposits of β-amyloid into plaques and intracellular formation of hyperphosphorylated tau into tangles), scientists have generated various animal models of AD ranging from a tiny fruit flies to the huge non-human primates. Despite countless efforts, recapitulation of the full-blown human AD in animals has been challenging due to non-identical phylogenetics, anatomy, and neuropathology between humans and these animals. However, generation of various rodent models (transgenic and non-transgenic) provides useful insights into understanding the pathogenesis as well as evaluating various AD therapeutic approaches.

2.7.6.1. The mouse models

The mouse model of AD is an efficient AD model as it processes the validity, reliability, reproducibility, time- and cost-efficiency with potentially valuable therapeutic implications. The mouse genetics have been mapped entirely with 85% homologous identity with the human genome enabling scientists to investigate the genetic component of AD, which is particularly important in the investigation of familial AD (Dietrich et al., 1996). In addition, the application of behavioral testing (i.e. associative

and spatial learning and memory) and the ease of breeding give the mouse a great advantage of being a useful AD animal model. The mouse AD model can be achieved via genetic manipulation (transgenic) or natural accelerated aging (non-transgenic). Although these models have yet to produce a human full-blown AD pathology, they nevertheless offer valuable insights into the pathogenesis as well as therapeutic strategies of AD.

2.7.6.1.1. The non-transgenic mouse model:

Senescence accelerated mice prone strain (SAMP8) has directed attention into AD pathology study. Aging is a major risk factor in the human AD pathogenesis as the aging brains are not only "worn-out" but also unable to withstand insults compared to the young brains. At the behavioral level, these mice have shown impairments in various types of learning and memory such as spatial or associative memory, which can be tested using the maze or classical conditioning paradigms, respectively. At the molecular levels, these mice exhibit an accelerated aging accompanied by several AD-related neuropathological hallmarks such as intracellular and extracellular amyloid deposits, tau hyperphosphorylation, decreased acetylcholine level, and neuronal loss (Canudas et al., 2005, Tomobe and Nomura, 2009, Manich et al., 2011). In addition to these later pathological changes, the SAMP8 mice also exhibit increased oxidative stress status prior to any plaque deposits (Tomobe and Nomura, 2009). It is postulated that in human AD pathology, the oxidative stress event occurs long before any visible neuropathological hallmarks (e.g. amyloid plaques, neurofibrillary tangles), which renders neurons vulnerable to toxicity. Thus, given these characteristics of the SAMP8

mice, it seems that these mice are a useful model of AD. However, the disadvantage of this SAMP8 model lies in the fact that there is an observed increase in extracellular glutamate level which greatly presents ischemic dementia instead of AD dementia.

2.7.6.1.2. The transgenic mice model:

In addition to the SAMP8 mice, genetic manipulation of amyloid precursor protein (APP) and its processing has been commonly used in studying AD pathology, especially the familial type of AD. Several hypotheses have emerged explaining the pathogenesis of AD (e.g. mitochondrial dysfunction, oxidative stress, calcium homeostasis, cholinergic degeneration, amyloid imbalance, etc.) but the most favorable hypothesis is the amyloid hypothesis, which is postulated based on the prime neuropathological hallmarks. The amyloid hypothesis simply states that the accumulation of amyloid proteins into aggregates and plaques is the cause of AD (Hardy and Allsop, 1991, Hardy, 2009). Given this cornerstone view, genetic manipulations of the genes related to APP and its processing (i.e. PS1 and PS2) have offered a valuable tool in understanding AD pathology.

a) APP mutations

Mutations of APP genes at various sites can alter the production of amyloid proteins. For example, the Swedish mutation occurs at the site of β-secretase cleavage can alter the APP processing toward the amyloidogenic pathways or the Florida, Indiana mutations at the site of γ-secretase can modulate the release of amyloid proteins. The transgenic mouse Tg2576 carrying SweAPP mutation displays an increased production of amyloid proteins, enhanced amyloid deposits and impaired performance in spatial

learning and memory tasks such as Morris water maze (Hock et al., 2009). Thus, these impairments are parallel to humans with AD pathology. However, these mice do not display the tangle formation and neuronal loss, which are other prime pathological changes in humans.

b) Double (APP/PS1) mutations

In order to reproduce similar pathological events observed in human AD, mutations in the APP and PS1 genes have been introduced into mice for generating AD model. Presenilin is a component of the γ-secretase complex that plays an important role in driving APP processing toward the amyloidogenic pathway. Introduction of both APP and PS1 transgenes reproduces phenotypes closely observed in humans. The AD transgenic mice were produced by crossing Tg1576 carrying APP mutation (K670N, M671L) with mice carrying PS1 mutation (M146L). At 3 month of age, these mice progressively develop amyloid deposits in various brain regions, especially areas that are vulnerable to AD pathology such as the hippocampus, and the amyloid deposits reach plateau at 12 months of age. These mice also displayed learning and memory impairment in various tasks from object recognition to Morris water maze paradigms. However, similar to the SweAPP transgenic mice, the APP/PS1 mice do not display neurofibrillary tangle or progressive neuronal death (Holcomb et al., 1998).

Thus, both the SweAPP and APP/PS1 transgenic mice do not reproduce the full complexity of human AD. In addition to cognitive impairment, AD patients experience various non-cognitive symptoms such as psychosis and depression, which terribly disturb their quality-of-life. The non-cognitive functions of these mice are not evaluated

making these models far from representing the human AD. However, these models provide useful information for evaluating therapeutic approaches targeted at reducing amyloid production or deposits to prevent memory impairment and preserve synaptic integrity and transmission.

2.7.6.2. The rat models

2.7.6.2.1. I.c.v. infusion of amyloid peptides

Exogenous administration of Aβ in rats produces a model that exhibits several neuropathological and behavioral features of AD useful for studying specific Aβ pathogenesis. Analogous to AD transgenic mice models, exogenous Aβ infusion does not represent the full spectrum of AD pathological features seen in humans. Although some previous studies show that injection of amyloid peptides did not display AD pathology (Games et al., 1992, Podlisny et al., 1992), other studies reveal that continuous infusion of Aβ in rats creates an AD-like pathology model (Nitta et al., 1994, Srivareerat et al., 2009). Indeed, these Aβ-infused rats show memory impairment in behavioral paradigms such as the MWM and passive avoidance (Nitta et al., 1997, Itoh et al., 1996). At the molecular and cellular level, the disrupted behavioral performance translates to failure of synaptic plasticity and cholinergic dysfunction (Itoh et al., 1996, Itoh et al., 1999, Ikeda et al., 2000). Additionally, some studies report neuronal loss and gliosis near Aβ deposits in the parenchyma after exogenous administration of amyloid species such as $A\beta_{40}$ and/or $A\beta_{42}$ (Stephan and Phillips, 2005). However, this disagreement is likely due to the potency of injected amyloid proteins, the injection route, and the length of treatment. Studies from our lab have shown that a concentration of 160 pmol/day of

$A\beta_{1\text{-}42}$ does not produce AD symptoms (Tran et al., 2010, 2011) while a concentration of 300 pmol/day of a mixture of $A\beta_{1\text{-}40}$: $A\beta_{1\text{-}42}$ in a 1:1 ratio reproduces AD pathology (Srivareerat et al., 2009, Alkadhi et al., 2011).

2.7.6.2.2. Cholinergic system dysfunction

Back in the late 60s and early 70s, biochemical modifications in brains of AD patients were examined with the hope of finding neurochemical aberrations involved in AD pathology. Further studies came along in support of this perspective reporting significant deficits of choline acetyltransferase (ChAT) levels (Bowen et al., 1982), an enzyme involved in acetylcholine (ACh) synthesis. Also, others began to address the reduction of choline uptake (Rylett et al., 1983, Rodriguez-Puertas et al., 1994), and ACh release (Nilsson et al., 1986) in pathological brains. Thus, given the important role of ACh in learning and memory and now AD pathology (Drachman and Leavitt, 1974, Gold, 2003), these studies began to form a cholinergic hypothesis of AD. With this in mind, several paradigms that resemble cholinergic dysfunction or lesions in the basal forebrain have been adopted as AD models (Toledano and Alvarez, 2004). Cholinergic lesion can be achieved acutely, via electrocoagulation (Kiyosawa et al., 1989), neurotoxin administration (e.g. ibotenic acid or quisqualic acid) (Monzon-Mayor et al., 2000), and fimbria/fornix transection (Krugel et al., 2001), or chronically, via i.c.v. infusion of quinolinic acid (Yamada et al., 1990). Even though these cholinergic dysfunction paradigms do not produce AD neuropathological hallmarks, they are valuable for evaluation of pharmacologic treatments such as cholinergic drugs (Murray and Fibiger, 1986) and glutamate receptor antagonists.

2.7.6.2.3. Fibrillary tangles model

In addition to cholinergic lesion AD paradigms, neurofibrillary tangles-related models have been developed as a potential alternative. The NFT-like models can be induced *in vitro* by up-regulating kinase activity and down-regulating phosphatase activity to produce hyperphosphorylation of tau (Lovestone and Reynolds, 1997). For instance, inhibition of protein phosphatase 2A by okadaic acid induces hippocampal AD-like tau hyperphosphorylation which in turn leads to spatial memory impairment in rats (Gong et al., 2000, Yin et al., 2010).

2.8.7. Alzheimer's disease, hippocampus, and memory

The hippocampus is the first brain region affected by AD pathology (Padurariu et al., 2012). Given the critical role of hippocampus integrity in learning and memory process, it is predictable that one of the prime symptoms of AD patients is spatial memory loss. Findings from both animal and clinical studies reveal that AD pathology not only deteriorates spatial (hippocampus-dependent) memory but also destroys non-spatial (hippocampus-independent) memory (Henderson et al., 1989, Tales et al., 2005, Blackshear et al., 2011, Ardiles et al., 2012). Accumulation of amyloid proteins in the hippocampus leads to synaptic and circuit disruptions (Reilly et al., 2003, Doherty et al., 2013), which eventually result in neuronal death (Abeti et al., 2011) and manifest as progressive memory impairment (Reitz et al., 2009). Indeed, *in vivo* bioimaging technique has demonstrated that the hippocampus is the critical region where the amyloid aggregation first disrupts hippocampal neuronal network (Sepulcre et al., 2013); thus making hippocampal neurons extremely vulnerable to insults. For example,

injection of fibrillar amyloid proteins in the hippocampus produce severe axonal damage in addition to infiltration of amyloid aggregates into mitochondria, disruption of subcellular structure and production of neuronal oxidative stress (Rosales-Corral et al., 2012).

2.8. Current treatments of Alzheimer's disease

Due to the multi-factorial etiology of AD, the disease has no current cure. Only two classes of drugs are clinically approved for treating the symptoms of AD, not the underlying causes. The first class is acetylcholinesterase (AChE) inhibitors which are effective in treating mild to moderate AD in only half of the patient population. In the central nervous system (CNS), ACh acts a neuromodulator which plays a role in plasticity, arousal, reward, and learning and memory processes (Auerbach and Segal, 1996, Colgin et al., 2003, Power et al., 2003, Mohapel et al., 2005). Disruption of the basal forebrain cholinergic system via exogenous neurotoxin administration results in cognitive impairment (Olton, 1990), which has been used as a novel AD model. Several studies report that AD slows the production of ACh in the nucleus of Meynert and substantially reduces the activity of AChE (Mufson et al., 2003, Herholz et al., 2004). Additionally, ACh receptors and choline acetyl transferase (ChaT), a critical enzyme for ACh synthesis, both decrease significantly in AD patients (Strong et al., 1991, Wevers et al., 2000). Therefore, treatment of AD patients with AChE inhibitors temporarily alleviates this cholinergic dysfunction, increasing the availability of ACh and in turn improving cognitive function.

Another class of drugs that treat the symptoms of AD is NMDA receptor antagonist (e.g. memantine), which is moderately efficacious in medium and severe AD. NMDA receptor activation plays an important role in hippocampal-mediated spatial learning and LTP. Blockade of hippocampal NMDA receptors by i.c.v. injection of d-2-amino-5-phosphonopentanoic acid (AP-5), a competitive NMDA receptor antagonist or genetic knock-out of NMDA receptor impairs learning and memory (Cui et al., 2004, Elvander-Tottie et al., 2006). Indeed, it has been shown that Aβ peptides can exert its toxic effect by disrupting hippocampal LTP via NMDA receptor-mediated pathway (Yamin, 2009). Moreover, studies have shown that in AD, NMDA receptor activity is distorted, which can lead to aberrant calcium regulation, which eventually results in excitotoxicity (Kuchibhotla et al., 2008). Hence, blocking the excitotoxicity pathway improves learning and memory in AD patients (Miller, 2007).

Furthermore, several alternative treatments have been proposed as potential therapeutic interventions for AD. Targeting the amyloid cascade hypothesis, most research focuses on the β and γ secretase inhibitors. β-secretase seems to be an appealing pharmacologic target since it catalyzes the rate-determining step in Aβ production. However, the progress toward a viable β-secretase inhibitor has been slow due to the predicament of designing a single molecule that possesses all the required pharmacological properties (Ghosh et al., 2008). In contrast, therapeutic intervention using γ-secretase inhibitors has advanced at a faster pace with several drugs in phase II and phase III clinical trials (i.e. begacestat, segamacestat, BMS-708163) (Panza et al., 2010). In addition, oral administration of γ-secretase inhibitors significantly improves

synaptic function in a model of AD transgenic mice which correlates positively with Aβ load reduction (Townsend et al., 2010). Unfortunately, γ-secretase is not exclusively selective for APP, but it also processes Notch, a protein involved in normal T-cell development. Thus, inhibition of the enzyme may cause serious gastrointestinal problems, reduced lymphocyte production, thymus atrophy, and hair color modification (Panza et al., 2010). With this in mind, several γ-secretase inhibitors standing at Phase III clinical trial have been halted. However, if the APP pathway can be selectively targeted in γ-secretase inhibitors treatment, it could be a promising therapy of AD pathology (Schor, 2011).

2.9. Regular exercise and memory

Regular exercise is a non-pharmacological approach that ameliorates memory impairment secondary to brain injury, enhances cognitive function, and prevents memory decline in the aged brain (Nichol et al., 2007, Kim SE, 2010). In addition to animal studies, epidemiological analysis in humans suggests that regular exercise can act as a preventive measure against cognitive disorders (e.g. amnesia, dementia) with minimal cost and adverse effects (Sofi et al., 2011). The neuroprotective effects of exercise are thought to be mediated by various molecular mechanisms including upregulation of neurotrophins (e.g. BDNF) and other molecules associated with learning and memory function (e.g. CaMKII, calcineurin), which in turn enhance synaptic plasticity (LTP) and improve performance in memory tasks. Exercised animals performed better in the spatial memory tasks (e.g. MWM or RAWM) compared to sedentary animals as indicated by shorter swim paths or fewer errors to find the target platform

(Luo CX, 2007, Nichol et al., 2007, Grace et al., 2009, Khabour et al., 2010). Similarly, exercise can also modify non-spatial memory as tested in the passive avoidance paradigm and object recognition tasks (Alaei et al., 2006, Albeck et al., 2006, Radak et al., 2006, Nichol et al., 2007, Grace et al., 2009, Hopkins and Bucci, 2010, Kim SE, 2010). Additionally, treadmill exercise restores memory impairment in rats treated with alcohol (Helfer et al., 2009), streptozocin (Reisi et al., 2009), reserpine (Aguiar et al., 2009), or sleep deprivation (Zagaar et al., 2012).

Even though the pronounced effects of exercise on cognition are well-supported throughout the literature, the exact exercise regime that can bring about the maximal benefits of exercise remains elusive. Although the training protocols vary drastically from each other, the majority of the studies indicate that exercise, despite the regimen, can be a potent non-pharmacological approach to preventing or treating cognitive impairment. The following section will briefly evaluate these studies while focusing on the type (forced vs. voluntary), duration, and intensity of exercise.

2.9.1. Types of exercise: voluntary vs. forced paradigms

Two exercise paradigms are often used in animal research: 1) Voluntary exercise, where animals are given access to running wheels *ad libitum* but housed singly; and 2) Forced exercise which involves the animals either running on a treadmill with a mild foot shock, a form of encouragement for continuous running or forced swimming in an enclosed pool. Both paradigms resemble human exercise patterns in certain ways. For example, voluntary exercise represents those who exercise at their own convenience whereas forced exercise represents people who must exercise in order to gain benefits

from exercise (i.e. post-stroke training, dementia prevention program). Forced exercise offers a definitive method for quantification allowing for comparison among protocols. On the other hand, despite the distance and length of time spent running, voluntary exercise seems to be more beneficial than forced running in laboratory rodents (Leasure and Jones, 2008, Yuede et al., 2009) as the rodents have control of when to run and do so in absence of a foot shock. However, voluntary exercise can produce isolation stress due to the condition of singly housed animals. In contrast, there is one human Parkinson's disease study showing that forced but not voluntary pedaling produces motor function improvement in affected patients (Ridgel et al., 2009). Despite these apparent differences, both voluntary and forced exercise modify metabolic proteins (Kirchner et al., 2008) and hippocampal mossy fiber sprouting (Toscano-Silva et al., 2010) in addition to increasing neurogenesis in the DG (Leasure and Jones, 2008). All of these processes are implicated in synaptic plasticity as well as learning acquisition and memory. In turn, these structural changes at the synaptic level lead to functional alterations in brain micro-circuitry which eventually manifests as improvement in memory-dependent tasks. It is interesting to note the existence of a possible ceiling effect of exercise that is species and life-style specific as wild-caught wood mice given free access to running wheels do not exhibit any enhancement in neurogenesis (Hauser et al., 2009).

Despite the inherent stress of treadmill exercise, forced running is more neuroprotective than forced swimming which has largely produced inconsistent results. Post-ischemic forced swimmers show a decline in cell proliferation in the DG of the

hippocampus but a better enhancement of spatial memory in the MWM (Luo et al., 2007). Under the same circumstances, Toldy et. al reported that regular swimming ameliorates the severity of NMDA lesions by reducing the DNA binding activity of inflammatory transcription factors NF-κB and activated protein-1 (AP-1) (Toldy et al., 2009). Furthermore, studies in human subjects show that aerobic exercise *per se* or in combination with non-aerobic exercise can contribute to cognitive improvement in aged or demented elderly (Heyn et al., 2004) and in AD patients (Rolland et al., 2010).

2.9.2. Exercise regimen: mild, moderate, and high intensity

Various protocols report the beneficial effects of exercise on cognitive function with different training duration and intensity. Current consensus views the beneficial effects of exercise on cognitive function as being characterized by an inverted U-shape curve in which too much or too little exercise is not beneficial for the brain health (Kamijo et al., 2004). In other words, there is a certain threshold where cognitive improvement becomes evident (beneficial point) yet upon passing this beneficial point, exercise can be deleterious. The deleterious effect of exercise could be due to fatigue that occurs with high intensity exercise; as this can eventually lead to potentially harmful mal-adaptations. For example, one study reports that low intensity exercise significantly retains spatial learning and memory while high intensity even impairs this ability (Kennard and Woodruff-Pak, 2012). On the other hand, chronic exercise of moderate intensity improves cognitive performance and prevents emergence of dementia, especially in youth with physical disability (Ploughman, 2008).

It is well-documented that regular exercise throughout a life-time can contribute greatly to cognitive gain in cases of aged, damaged, or pathological brains. However, detraining may reverse the neuroprotective effect of exercise as BDNF levels fall back to normal (Berchtold et al., 2010) or even below the basal levels (Radak et al., 2006).

2.10. Possible mechanisms that mediate the effect of exercise on the AD brains:

The beneficial effects of regular exercise on mental health can be mediated by various neurophysiological pathways in which alterations in cellular and molecular signaling pathways can affect the supramolecular mechanisms (i.e. neurogenesis, angiogenesis, and synaptogenesis) and eventually leading to memory improvement seen at the behavioral level.

2.10.1. The supra-molecular mechanisms:

2.10.1.1. The vasculature and angiogenesis

One possible mechanism of the beneficial effect of exercise on cognition is the improvement in the cerebral vasculature. Several studies showed that acute exercise in human (Smith et al., 2010) and chronic exercise in rats and monkeys (Swain et al., 2003, Rhyu et al., 2010) increased cerebral blood flow and volume. Cerebral vascular manipulations (i.e. angiogenesis, vasodilation) can alter the process of learning and memory. Much evidence shows that regular exercise improves not only brain vasculature in healthy young and aged brains (Ding et al., 2006b, Bullitt et al., 2009) but also in the pathological brains (i.e. Parkinson's disease) (Al-Jarrah et al., 2010). Regular exercise enhances cerebral vasculature structure and function in various areas of the brain including the cerebellum (Black et al., 1990, Isaacs et al., 1992), motor cortex

(Kleim et al., 2002, Swain et al., 2003), striatum (Clark et al., 2009, Al-Jarrah et al., 2010), and hippocampus (Ekstrand et al., 2008, Van der Borght et al., 2009). Exercise seems to improve cognitive function by up-regulating angiogenic factors such as vascular endothelial growth factor (VEGF), angiopoetins, and insulin-like growth factor 1 (IGF-1) (Trejo et al., 2001, Ding et al., 2006a, Ding et al., 2006b) which have been implicated in vascular growth.

Angiogenesis, the formation of new blood vessels, is one of the supramolecular mechanisms proposed to occur during exercise. The cerebral vasculature is highly plastic through life and plays an important role in the brain metabolism. Thus, it is of speculation that angiogenesis can help to extend the cerebral vasculature by bringing new nutrients to deprived neurons and increasing the brain blood perfusion and increasing the chance of survival of dying neurons due to depleted energy. It has been observed that the AD brains experience a high degree of hypoperfusion as well as reduced metabolic activity (Aliev et al., 2003, Johnson et al., 2005). In contrast, regular exercise stimulates cerebral angiogenesis via the action of vascular endothelial growth factor (VEGF) which promotes cerebral perfusion leading to better memory performance (Al-Jarrah et al., 2010, Tang et al., 2010).

2.10.1.2. Neurogenesis

Neurogenesis, the birth of new neurons, is a key mechanism in unraveling the important role of exercise on cognition. Hippocampal neurogenesis takes place in the dentate gyrus of the hippocampus exclusively. Several studies report that exercise increases hippocampal cell proliferation (Ra et al., 2002, Uda et al., 2006, van der Borght

et al., 2006, Van der Borght et al., 2009) under menopausal conditions (Jin et al., 2008), in case of brain insults (Redila and Christie, 2006, Jin et al., 2010, Leasure and Nixon, 2010), and in stressed or aged animals (Nakajima et al., 2010, Kannangara et al., 2011). Additionally, dentate gyrus granule cell survival, proliferation, and maturation rates are significantly enhanced in exercised animals compared to their matched controls, and are positively correlated with the degree of neuroprotection (Leasure and Jones, 2008, Snyder et al., 2009). Furthermore, other findings show that exercise can elevate both cell proliferation and survival in exercised animals (van Praag et al., 1999, Wu et al., 2008, Jin et al., 2010). Interestingly, maternal exercise during gestation can also reinforce the pups' cognitive function by enhancing hippocampal cell survival (Kim et al., 2007). Even though it is not clear how neurogenesis can be beneficial to the brain, it is assumed that these new neurons can incorporate themselves into existing functional circuits and function along with other neurons (Ge et al., 2008). If this is true, neurogenesis can help to replace old neurons in order to maintain normal cognitive function. Also, the AD brain suffers a tremendous loss of neurons in areas critical to learning and memory (i.e. hippocampus, basal forebrain); thus, exercise-induced neurogenesis may contribute to the beneficial effect of exercise on AD.

2.10.1.3. Synaptogenesis

While the effect of exercise on neurogenesis has been studies with various techniques, its effect on synaptogenesis is under-investigated. This is possibly due to the fact that synaptogenesis is often associated with the process of learning (Kleim et al., 1996, Ramirez-Amaya et al., 1999), which makes it hard to extrapolate the synaptic

changes are caused solely by exercise. At the same time, biased quantification of stereology in studying synaptogenesis makes it uncommon compared to neurogenesis investigation. Further study is needed in order to distinguish whether the exercise-induced morphological changes of the synapses are permanent, since the effects have been reported to be transient after spatial learning (Eyre et al., 2003). However, accumulating evidence shows the positive effect of exercise on synaptic modifications. For example, voluntary exercise can add to dendritic complexity, density, and length (Eadie et al., 2005, Redila and Christie, 2006, Stranahan et al., 2009) and increase the number of dendritic spines (Dietrich et al., 2008). In addition to changes in the cytoarchitecture, exercise modulates pre- and post-synaptic components that play an important role in synaptic plasticity. For instance, regular exercise increases the level of synaptophysin, a vesicular protein that regulates synaptic transmission, and calcium/calmodulin-dependent protein kinase II (CaMKII) (Hescham et al., 2009). In addition to neurotrophic effects, voluntary exercise has been shown to cause the generation of new oligodendrocytes, a trophic factor (Krityakiarana et al., 2010) despite the fact that the central nervous system (CNS) does not easily regenerate. Assuming the neuronal loss of AD pathology will reduce the number of synapses, exercise-induced synaptogenesis can be an important mechanism of exercise on AD brain.

2.10.2. The molecular signaling pathways:

The inhibitory effect of AD pathology on the synapses might be explained by deleterious changes in key signaling pathways while regular exercise seems to prevent these changes.

2.10.2.1. The energy dependent mechanism:

The AD brains exhibit a reduced metabolism probably owning to the reduced energy supply as hypoxia is observed in AD brains (Peers et al., 2007). In contrast, regular exercise is known to produce ATP, a potent vasodilator that overcomes the sympathetic activated vasoconstriction, which in turn can increase oxygen supply to the local tissues (Bangsbo et al., 2001, Campbell-O'Sullivan et al., 2002). Mitochondria are dynamic organelles considered to be the major power horse of the cell. They are also important in the regulation of calcium homeostasis and apoptosis (Pinton et al., 2008, Grimm, 2012). It has become increasingly evident that mitochondrial dysfunction is one of the primary events in the early stages of AD (Maruszak and Zekanowski, 2011, Ye et al., 2012) and has the tendency to worsen as the disease progresses. Mitochondrial dysfunction is not only restricted to the brain regions but also has been found in fibroblasts and blood cells of AD patients (Gibson et al., 1997, Gibson et al., 1998, Devi et al., 2006, Valla et al., 2006, Wang et al., 2008b) suggesting a systemic state of deterioration in the disease. Furthermore, AD brains display a higher ratio of dysfunctional mitochondria compared to normal mitochondria as well as a reduction in mitochondrial energy-balancing molecules such as mitochondrial uncoupling protein 2 (UCP2), and ubiquitous mitochondrial creatine kinase (uMtCK) (Santos et al., 2010, Garcia-Escudero et al., 2013). Models of AD also indicated the presence of impaired mitochondrial biogenesis, its axonal transport and dynamics (Calkins et al., 2011, Sheng et al., 2012). In contrast, exercise is known to produce a positive effect on mitochondrial population and function. For example, exercise induced mitochondrial biogenesis

extensively in human skeletal muscles (Wang et al., 2011, Bartlett et al., 2013) and mice brains (Steiner et al., 2011) making exercise an appealing target for novel treatment of CNS diseases, which are usually accompanied by mitochondrial dysfunction. Since the mitochondrial hypothesis of AD postulates that inheritance sets the mitochondria baseline in term of function and durability, the pathology produces compromised mitochondrial function along with neuronal death (Swerdlow and Khan, 2004, Swerdlow et al., 2010). Thus, probably by increasing the baseline set point of these AD mitochondria, regular exercise makes them more resistant to insults (e.g. amyloid toxicity).

2.10.2.2. The energy-independent mechanisms:

2.10.2.2.1. The effects of exercise on non-BDNF neurotrophins:

a) Nerve growth factor (NGF)

Many studies have shown the effect of exercise on cognition via a BDNF-related mechanism. Nevertheless, the definitive factors constituting the beneficial effect of exercise on cognition remain unclear; one of these factors maybe the nerve growth factor (NFG). NGF also belongs to the same neurotrophin family as BDNF and binds to transmembrane tyrosine kinase receptors, but shows preference toward the TrkA receptor subtype (Chung et al., 2010). NGF signaling is the main neurotrophic support of cholinergic neurons in the basal forebrain, a critical structure for learning and memory that is severely affected during AD pathology (Capsoni et al., 2010). In addition to promoting neurogenesis, NGF can prevent apoptosis and neurite growth upon receptor activation and dimerization (Jang et al., 2007). TrkA immunoreactivity is modified in the

Cornu Ammonis-1 (CA1) hippocampal sub-region by ischemic forebrain injury (Hwang et al., 2005); however, post-ischemic administration of the viral vector carrying the NGF gene stalls the cell death (Shirakura et al., 2004). Exercise appears to increase the level of NGF and TrkA proteins (Matsuda et al., 1991, Neeper et al., 1996), which in turn leads to better motor performance in a model of brain ischemia (Chung et al., 2010). Other studies report that exercise can increase level of NGF and restore cognitive deficits in a model of streptozotocin-induced diabetic and aged rats (Chae et al., 2009, Chae and Kim, 2009, O'Callaghan et al., 2009). Hence, NGF could serve as another possible mechanism for exercise's beneficial effect on cognitive function.

b) Other trophic factors:

The levels of glial cell line-derived neurotrophic factor (GDNF) are also up-regulated during exercise training in both young and aged animals (McCullough et al., 2013). The exercise-induced GDNF increase can also be attributed to the neuroprotection in a model of Parkinson's disease (Tajiri et al., 2010, Lau et al., 2011). Additionally, human mesenchymal stem cells, transfected with GDNF is able to protect cerebral ischemic rats from injuries, which suggests a novel potential treatment of stroke (Horita et al., 2006). However, further functional studies about GDNF are needed before concluding that GDNF may play a role in exercise-induced memory performance.

Also, insulin-like growth factor 1 (IGF-1) is a potent trophic factor that can act peripherally and centrally (Hiney et al., 1996, Daftary and Gore, 2005). IGF-1 can interact with VEGF to mediate angiogenesis. Both IGF-1 and VEGF levels are known to increase during exercise (Voss et al., 2013). At the same time, IGF-1 can modulate the action of

exercise-induced BDNF (Cassilhas et al., 2012). Thus, it is postulated that IGF-1 maybe the mediator enabling the effect of exercise on brain health via BDNF-related mechanism (Trejo et al., 2001).

2.10.2.2.2. The effect of exercise on neurotransmitters

Acetylcholine (ACh) is an important neurotransmitter that participates in various brain functions such as cerebral blood flow regulation, learning and memory and synaptic plasticity. The major site of ACh production in the brain is the Nucleus of Meynert, where AD pathology severely destroys its integrity. As a result, AD patients showed progressive cholinergic denervation and reduced ACh release much owning to the neuronal loss in the basal forebrain. In contrast, regular exercise has been shown to enhance ACh-induced vasodilation, which in turn results in better behavioral performance (Johnson et al., 2001). It is thought that ACh may module synaptic plasticity via the action of BDNF and/or GDNF (Vianney et al., 2013). Thus, exercise-induced positive regulation of both ACh and BDNF may ameliorate the AD brains and produces better memory performance seen in behavioral tasks.

In AD, it is observed that there is a significant decrease of norepinephrine (NE) and its transporter in the locus coeruleus of the brainstem (Tejani-Butt et al., 1993, Heneka et al., 2010). This NE deficiency is highly correlated with the high cortisol level and the progression of AD as well as hippocampal atrophy (Davis et al., 1986, Hartmann et al., 1997, Lupien et al., 1998). However, regular exercise can increase the level of circulating NE which is considered to offset the deleterious NE reduction seen in AD (Berkin et al., 1988).

In addition, regular exercise is known to increase the level of serotonin (5-HT) and dopamine (Young, 2007). At the same time, anandamine, an endocannabinoid that regulates dopamine release, is secreted during exercise training resulting in activation of the endocannabinoid system (Sparling et al., 2003, Dietrich and McDaniel, 2004). AD patients show impairment in classical conditioning paradigm testing for associative learning, which is a type of serotoninergic modulation (Woodruff-Pak et al., 1996, Hamann et al., 2002). Thus, by improving the 5-HT signaling, exercise is thought to ameliorate the memory impairment associated with AD.

2.10.2.2.3. The effect of regular exercise on glucocorticoid signaling pathway

The hypothalamic-pituitary-adrenal (HPA) axis activity is an important regulation of the stress response. The arrival of a perceived stress directly activates the hypothalamus, which will cause the release of corticotrophin releasing hormone (CRH) from the CRH neurons in the hypothalamus paraventricular nucleus (PVN) and the release of vasopressin (AVP). CRH then can activates the anterior pituitary leading to secretion of ACTH whose action can be potentiated by AVP when releasing together in the portal capillaries. ACTH can travel via the blood to the adrenal cortex causing the release of glucocorticoid (GC) (cortisol in humans and corticosterone in animals). It has been shown that GC level and memory are linked in an inverted U relationship where too high GC level is actually detrimental to memory (Martinez and Kesner, 2009). Numerous studies establish that AD patients exhibit an elevated cortisol level compared to age-matched controls, which correlate with their clinical progression of AD and hippocampal atrophy (Davis et al., 1986, Lupien et al., 1998, Csernansky et al., 2006). In

contrast, regular exercise is known to normalize the elevated corticosterone levels in sleep-deprived rats (Vollert et al., 2011). Together, these data signify that regular exercise can lower GC level returning it to normal concentration so that exercise is beneficial in AD. One of the proposed mechanisms of this action is that exercise training can produce GC globulin which can scavenge circulating GC (Droste et al., 2009).

2.10.2.2.4. The effect of regular exercise on oxidative stress

Accumulation of reactive oxygen species (ROS) is seen with aging, oxidative stress related diseases, and neurodegenerative diseases. Excessive amounts of ROS formation can overwork DNA repair mechanisms and mitochondrial antioxidant defense systems in addition to altering nucleic acids, membrane phospholipids, and proteins, which eventually lead to oxidative stress-induced apoptosis. It appears paradoxical that exercise can effectively protect the brain while at the same time, the production of ROS gradually increases during exercise. This paradox can be explained by the "hormesis theory" in which low level of toxin exposure benefits the organism (Radak et al., 2005). Exercise of moderate intensity is thought to harmonize the ROS balance and reduce oxidative stress (Goto et al., 2004), whereas intense exercise or a single bout of exercise induces oxidative damage (Rietjens et al., 2007, Teixeira et al., 2009). However, other report shows that strenuous and over-training exercise does not cause oxidative damage to the brain since chronic training can lead to modifications such as oxidative neuro-adaptation (Ogonovszky et al., 2005).

Compared to controls, the AD brains are present with increased levels of lipid peroxidation and protein oxidation products (e.g. advance glycation end products,

4HNE, carbonyls) and DNA oxidation (e.g. 8-OHdG) with high level of metal ions (e.g. Fe, Cu, Hg, Al) (Sultana et al., 2006). 4HNE can reduce the activity of Na/K ATPase pump by binding to the sulfhydryl group, which in turn can alter calcium homeostasis (Liu et al., 2012). In contrast, regular exercise is known to up-regulate the expression of antioxidant enzymes that can counteract the oxidative damage caused by AD pathology. For example, the level of glutathione transferase that degrades 4HNE is highly increased during exercise training (Reddy et al., 1995)

Another mechanism by which exercise can counteract the detrimental effect of oxidative stress on the brain is to increase the activity of proteasomal, mitochondrial antioxidant defense mechanisms (superoxide dismutases (SOD), glutathione peroxidase (GSH-Px), and catalase) and DNA-repair enzymes (8-oxoG-DNA glycosylase (OGG1)), whose primary function is the protein quality control. Mitochondrial defense is significantly enhanced after exercise training in the brains of diabetic animals (Ozkaya et al., 2002) or Alzheimer's disease (Um et al., 2008). Somani and colleagues have shown that the brain antioxidant system is distinctly regulated in different brain areas. For example, SOD activity notably increases in the corpus striatum and brainstem regions with exercise, whereas hippocampal SOD activity is low. However, GSH-Px activity is enhanced the most in the hippocampus (Somani et al., 1995). Moreover, other studies have shown that exercise decreases oxidative markers such as reactive carbonyl derivatives (RCDs) (Ogonovszky et al., 2005, Szabo et al., 2010) and free radical elements (Radak et al., 2006).

2.10.2.2.5. The effect of regular exercise on the Wnt signaling pathway

Recent study has shown that the Wnt pathway is involved in mediating the oligomeric amyloid peptide's toxicity via limiting cell proliferation (Shruster et al., 2011). In contrast, regular exercise has been shown to module this Wnt/β-catenin signaling pathway. For example, regular exercise can activate cacyclin binding protein (Cacycb), which can ubiquitinate β-catenin. Ubiquitination of β-catenin leads to proteasomal degradation of this protein, thus causing Wnt signaling modulation. Exercise-induced neurogenesis is postulated to be mediated by activation of CAMKα via Wnt/frizzled signaling (Varela-Nallar and Inestrosa, 2013). Thus, these findings suggest that exercise may exert a beneficial effect on the AD brain via the Wnt signaling pathway.

2.10.2.2.6. The effect of regular exercise on amyloid deposition, AD-related molecules, and excitotoxicity

Amyloid plaques are one of the neuropathological hallmarks required for definitive diagnosis of AD. Amyloid aggregates can exert a multiple role in toxicity involving oxidative stress induction, calcium dysregulation via amyloid channel formation and glutamate receptors, metabolic off-balance, and deleterious alterations in intracellular transduction pathways. In addition, Aβ aggregates can facilitate hyperphosphorylation of tau via modulation of kinases that phosphorylate tau (Park et al., 2012). Altogether, amyloid aggregate is proposed as a serious threat to not only normal neurons but seem to be a powerful killer of AD vulnerable neurons. In fact, the amyloid hypothesis remains to be the cornerstone for AD pathogenesis as amyloid aggregates can potentiate and render vulnerable neurons to death. Transgenic model of

AD showed an increased amyloid load and deposits while exercised transgenic mice displayed a significant reduction in these parameters. Thus, decreasing the amyloid burden is possible strategy that is employed by the non-pharmacological approach (e.g. exercise). In fact, AD/exercised animals displayed decreased levels of amyloid load and plaque deposition (Adlard et al., 2005, Um et al., 2008, Nichol et al., 2009, Yuede et al., 2009). The proposed mechanism of exercise-induced amyloid reduction is thought to be regulated via proteolytic modifications in APP processing (Adlard et al., 2005).

2.11. Exercise and anxiety-like behaviors

Not only does exercise provide a beneficial effect on cognitive function, but also it is effective as other pharmacological treatments or psychotherapies for anxiety and depressive disorders (Broman-Fulks et al., 2004, Broman-Fulks and Storey, 2008, Bartley et al., 2013). Numerous studies have established the beneficial relationship between exercise and psychological symptoms associated with aging (Thune-Boyle et al., 2012). In addition to the pronounce anxiolytic effect of exercise in humans, studies have shown that exercise can also prevent anxiety-like and defensive behaviors in rodents (Sciolino et al., 2012), even in aged animals (Pietrelli et al., 2012). Mice pups that are maternally deprived show reduced anxiety-like behaviors when the mothers exercise during the pregnancy (Uysal et al., 2011). The mechanism by which exercise can reduce anxiety is possibly by preventing the activation of neurons induced by stress (Schoenfeld et al., 2013).

2.12. Exercise, Alzheimer's disease, cognitive and non-cognitive functions

Given the opposite effects of exercise and Alzheimer's disease pathology on cognition and psychological behaviors, it is of great interest to investigate whether exercise can prevent cognitive and non-cognitive disturbances associated with AD. In this study, we have examined the impact of regular moderate treadmill on spatial memory, anxiety-like behaviors, synaptic plasticity and related signaling cascades in a rat model of sporadic AD.

3. MATERIALS AND METHODS

3.1. Animals and housing conditions

Adult male Wistar rats weighing 176-200g at the beginning of the experiments were purchased from Charles River Laboratories, Wilmington, MA. Upon arrival, rats were separated into groups of six and housed in Plexi glass cage in a climate-controlled room (25^0C) on a 12hr/12hr light/dark cycle and provided regular chow diet and water *ad libitum*. All animal experiments followed the instructions from National Research Council's Guide of The Care and Use of Laboratory Animals and with the approval of Institutional Animal Care and Use Committee at the University of Houston.

3.2. Animal manipulations:

In this study, after 7 days acclimatization period, rats were randomly assigned into 4 groups: control, exercise, amyloid-infused (Aβ), and amyloid-infused/ exercised (Ex/Aβ). Rats were manipulated according to the following schematic diagram (figure 18):

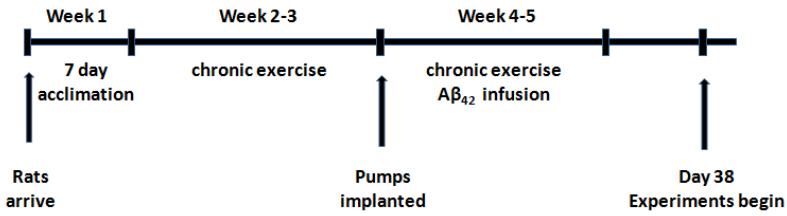

Figure 18. Experimental timeline for all animal manipulations.

3.2.1. Exercise training

Rats ran on a motorized rodent treadmill (Columbus Instruments) (Figure 19) between 9:00 am and 4:00 pm, 5 days/week for 4 weeks as described (Vollert et al., 2011, Zagaar et al., 2012, Dao et al., 2013). Before exercise training, all rats were

familiarized with the treadmill environment. Exercise and Ex/Aβ rats ran in sessions (15 minutes each) with a 5 minutes break in between the sessions to avoid muscle fatigue. During the first two weeks, rats ran 2 sessions at a speed of 10 m/min while during the 3rd and 4th weeks, rats ran 3 and 4 sessions respectively at a speed of 15 m/min. In order to encourage the rats to run continuously, the treadmill constantly delivered a mild foot shock (intensity = 0.5 mA) at the beginning of the running lanes. This shock appears to be mild and not stressful to the animals. In fact, the rats quickly learned to avoid the shock by staying on the running trails throughout the training sessions.

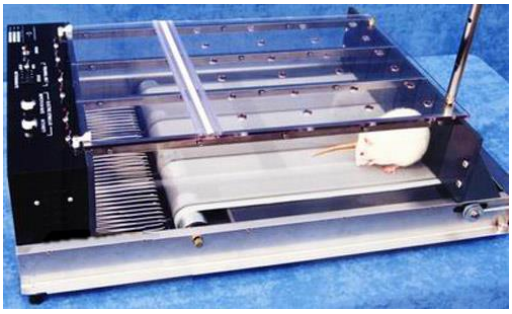

Figure 19. Columbus motorized rodent treadmill

3.2.2. Osmotic pump implantation

Two weeks after the acclimatization period, the control and exercise groups underwent a sham operation. Meanwhile, the Aβ and Ex/Aβ groups were implanted with osmotic mini-pumps (14 days duration) (Alzet, Cupertino, CA) containing $A\beta_{1-42}$ peptides (AnaSpec Inc., San Jose, CA). The peptides were dissolved in a solution containing 64.9% distilled water, 35% acetonitrile, and 0.1% trifluoroacetate (TFA) to prevent peptide aggregation in the pump. The pumps were assembled as instructed in the manufacture's manual, filled with $A\beta_{1-42}$ solution (250 pmol/day), and primed in isotonic (0.9% NaCl) saline at 37^0C overnight. Rats were i.p. injected with a mixture of

ketamine (75 mg/kg) and xylazine (2.5 mg/kg), which proved to be adequate for the implantation surgery duration (Webster Veterinary, Devens, MA). Then, the implantation site was shaved and cleaned with rubbing alcohol. The rat was stereotaxically framed with nose bar adjusted at 0.0. The skull was exposed by a 2.5 cm midline incision starting behind the eyes. The infusion cannula was implanted into the cerebral lateral right ventricle (AP: -0.3, L: 1.2, V: 4) and steadily fixed with dental cement. The pump was placed in a subdermal pocket in the back of the rat. The surgery site was closed with wound clips and kept aseptic with tincture of iodine, 60X diluted chlorohexidine, and triple antibiotic ointment. The rats were put back in their home cages and monitored until full recovery.

3.3. Behavioral assessments

3.3.1. Cognitive function testing

The radial arm water maze (RAWM), a hybrid of the Morris water maze and radial arm maze, is a useful test of learning of learning and memory function in rodents. It is a circular black water pool that consists of six swimming arms with an open central area (figure 20A). The experiments were carried out in a dimly lit room with visual cues placed on the surrounding walls. Each rat was randomly assigned a goal arm, which contained a hidden black platform located at the end of the arm. The test consists of twelve learning trials, one short-term and one long-term memory test. For each learning trial and memory test, the rat was randomly released at an arm different from the goal arm. The rat must then swim and locate the platform, which is submerged about 1 cm under the water. The rat was allowed a maximum time of 1 minute for each learning

trial or memory test. Quantification of learning and memory in the RAWM was done by counting the number of errors. An error was counted when the rat's entire body entered more than halfway into an arm that was not the goal arm or into the goal arm but failed to approach the platform. The number of errors ranges from 1 to a maximum of 7 as the rat can swim into 7 arms within 1 minute. When the rat failed to locate the platform within 1 minute, it was manually guided to the platform and automatically given a score of 7 errors. Once on the platform, the rat was allowed 15 seconds sitting time on the platform before the next learning trial. The timeline for all learning trials, short-term memory (STM), and long-term memory (LTM) tests were carried out as in the schematic representation in figure 20B.

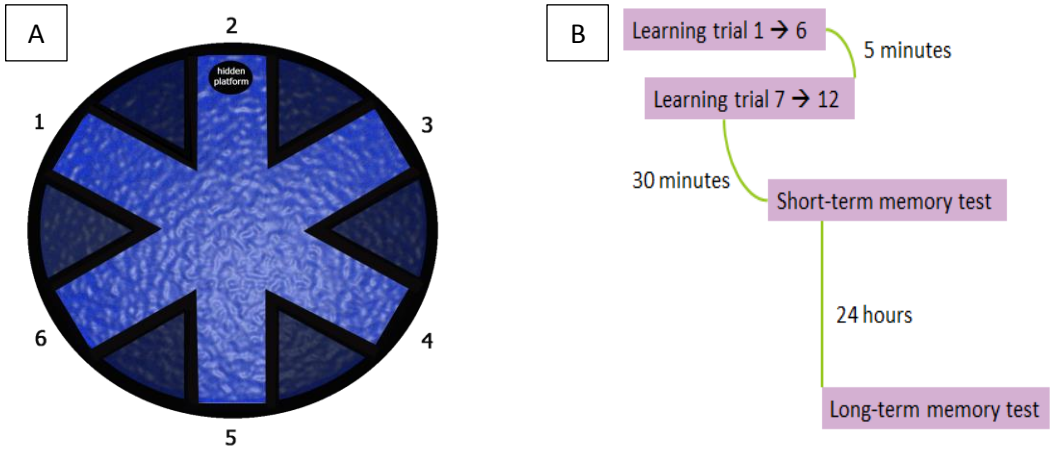

Figure 20. A) The radial arm water maze pool. B) Schematic representations of learning trials, short-term and long-term memory test in the RAWM. The animals received 5 minutes between two sets of learning trials as resting time. After the 12th learning trial the rats were returned to their home cage until they were tested for STM and LTM. The same rats were used for the learning trials, STM, and LTM tests.

3.3.2. Non-cognitive testing:

In order to investigate the effect of regular treadmill exercise on non-cognitive performance in rats with AD pathology, a battery of non-cognitive behavioral tests was done in the following order:

3.3.2.1. Open field (OF)

The OF task is a common test that can effectively evaluate anxiety level, exploratory behavior, and locomotor activity in rodents (Gould et al., 2009). The OF apparatus consisted of an open area (40 x 60 cm) surrounded by Plexiglas walls (50 cm height). A 25 x 25 cm area in the middle of the chamber is defined as the center zone. The rat was placed in the center of the chamber and allowed 30 minutes to explore the environment. The activity was detected by infrared light sensors and quantified by Opto-Varimex Micro Activity Meter v2.00 software (Optomax, Columbus Instruments; OH). Sensors are positioned to reveal a two-dimensional cage and monitor rearing (6 sensors/cage). Every movement was detected by beam breaks, which were recorded by the computer for further analysis. Each experiment was conducted with one rat per chamber and recorded in 3-minute test intervals. Total activity, total distance traveled, and percent time spent in the center area were analyzed. The OF apparatus was cleaned with 70% ethyl alcohol and aired out after each rat.

3.3.2.2. Light-dark (LD) box

The LD paradigm is validated as a sensitive test of disorders that involved generalized anxiety (Araujo et al., 2012). The LD box consists of a light compartment (27 x 27 x 27 cm) and a dark compartment (blackened walls and floor, 27 x 18 x 27 cm)

separated by a single partition with an opening (7 x 7 cm) that allows passage between the two compartments (Salim, et al., 2010). The apparatus was placed in an enclosed area of a behavioral room under standard lighting conditions with only one observer in the experiment room. The experiment lasted 5 minutes. Using a Microsoft Excel software, the observer reported data by manually enter “L” or “D” into the program. The experimenter entered “L” or “D” whenever the rat was in the light or dark compartment respectively. By doing so, the experimenter was able to calculate the time rat spent in light or dark compartment. When the experiment began, the experimenter put the rat in the center of the light compartment and immediately pressed “L” on the keyboard. This automatically started the computer clock. Whenever the rat moved to the dark compartment, a “D” was pressed. Entrance into the light or dark compartment was recorded when both front paws and shoulders were inside the compartment. Anxiolytic behavior was quantified by the time spent in the light compartment.

3.3.2.3. Elevated plus maze (EPM)

The EPM consists of two open and two closed arms (10 cm x 50 cm length) that intersect at the middle creating a plus shape. The EPM was elevated 50 cm above the ground and placed in an enclosed area of the behavioral core room. The EPM procedure was carried out as described (Xiang et al., 2011). Briefly, the rat was placed at the intersection area (10 x 10 cm) of the four arms and allowed to explore the apparatus for 5 minutes. The rat’s movements during the testing period were recorded by a digital camera that was connected to a computer for data collection. Time spent in the closed arms, center area, and total distance traveled were analyzed.

3.4*. In vivo* extracellular recordings:

Seventeen days after implantation surgery, *in vivo* electrophysiological recordings in the sub-regions Cornu Ammonis 1 (CA1) and dentate gyrus (DG) of the hippocampus were accomplished as described (Aleisa et al., 2006). Briefly, rats were anesthetized with urethane via i.p. injection (1.2 g/kg, Sigma Aldrich, USA). The anesthetized rats were placed in the stereotaxic frame (nose bar at 0.0). Wound clips were removed if any had remained from the implantation surgery. Similar to the pump implantation surgery, a 2.5 cm midline sagittal incision was made to expose the skull. Two holes were drilled for placing the stimulating and recording electrodes whose positions would be specified below. During recording, the rat was subcutaneously grounded with a wire wrapped in gauze soaked in 0.9% saline to prevent background noise.

CA1 recording: On the left side of the brain, a hole was drilled for placing the concentric bipolar stimulating electrode (twisted tephlon-coated stainless steel wires) into the CA3 region of the left hippocampus. The stimulating electrode was angled at 5^0 toward the midline in order to stimulate the Schaffer collateral/commissural pathway, which connects the CA3 to the CA1 sub-regions (AP: -3, L: 3.5, V: 2.8). Similarly, another hole was drilled on the right side of brain to place the capillary glass (1–5 MΩ) recording electrode into the CA1 region of the right hippocampus (AP: -3, L: 2, V: 2). The recording electrode was filled with 1% fast green dye in 2M NaCl in order to visually confirm the correct recording electrode position upon dissection at the conclusion of the experiment (see figure 21).

DG recording: On the right side of the brain, a hole was drilled for placing the concentric bipolar stimulation electrode to stimulate the perforant pathway via the angular bundle (AP: -8, L: 4.7, V: 1.2). The glass capillary recording electrode was placed into the DG area of the right hippocampus (AP: -3, L: 2, V: 3.5) (see figure 21).

The stimulating electrode was connected to a stimulator via an isolation unit (Grass Inst., USA). The recording electrode was connected to an amplifier system which connects to the computer for read-outs. Position of the electrode could be adjusted, if necessary, to obtain maximal responses.

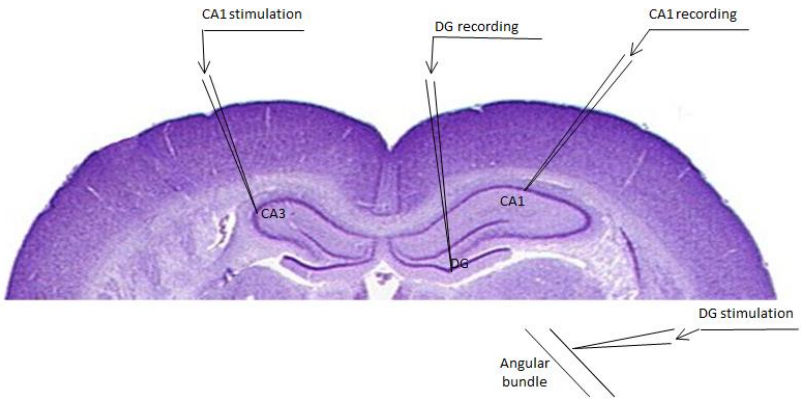

Figure 21. Positioning of the recording and stimulating electrodes for extracellular recording from CA1 and DG areas.

3.4.1. Basal synaptic transmission:

Once a robust response was found, rat was left without simulation for 30-minutes stabilization period. The input/output (I/O) curve was constructed by plotting various stimulus intensities versus the field excitatory post-synaptic response (fEPSP) slope, a measure of synaptic strength. The stimulus intensities at which the response was minimal or maximal constructed the first and last point of the I/O curve

respectively. The increment between the minimal and maximal points of the I/O curve was calculated by taking the difference between the maximal stimulus intensity and the minimum stimulus intensity and then divided by 7. We also recorded the voltages that required to evoke minimal, 30% of the maximum, and maximal response in each group.

3.4.2. E-LTP and L-LTP induction and recordings:

After the I/O curve recording, a 10 minute stabilization period was allowed without further stimulation. The test stimulus was adjusted to 30% of the maximal response. A baseline was established with test stimuli (1pulse/30s) to evoke population spikes (pspike) for 20 minutes. E-LTP was evoked by one train of HFS while multiple trains of HFS induced L-LTP as detailed in figure 22. Both E-LTP and L-LTP magnitudes were quantified by changes in the field excitatory post-synaptic potential (fEPSP) slope and pspike amplitude recorded for 1 hour or 5 hours respectively following repetitive stimulation. The fEPSP slope and pspike amplitude represent synaptic strength and the number of neurons reaching firing threshold respectively (Alzoubi et al., 2007, Alzoubi and Alkadhi, 2007).

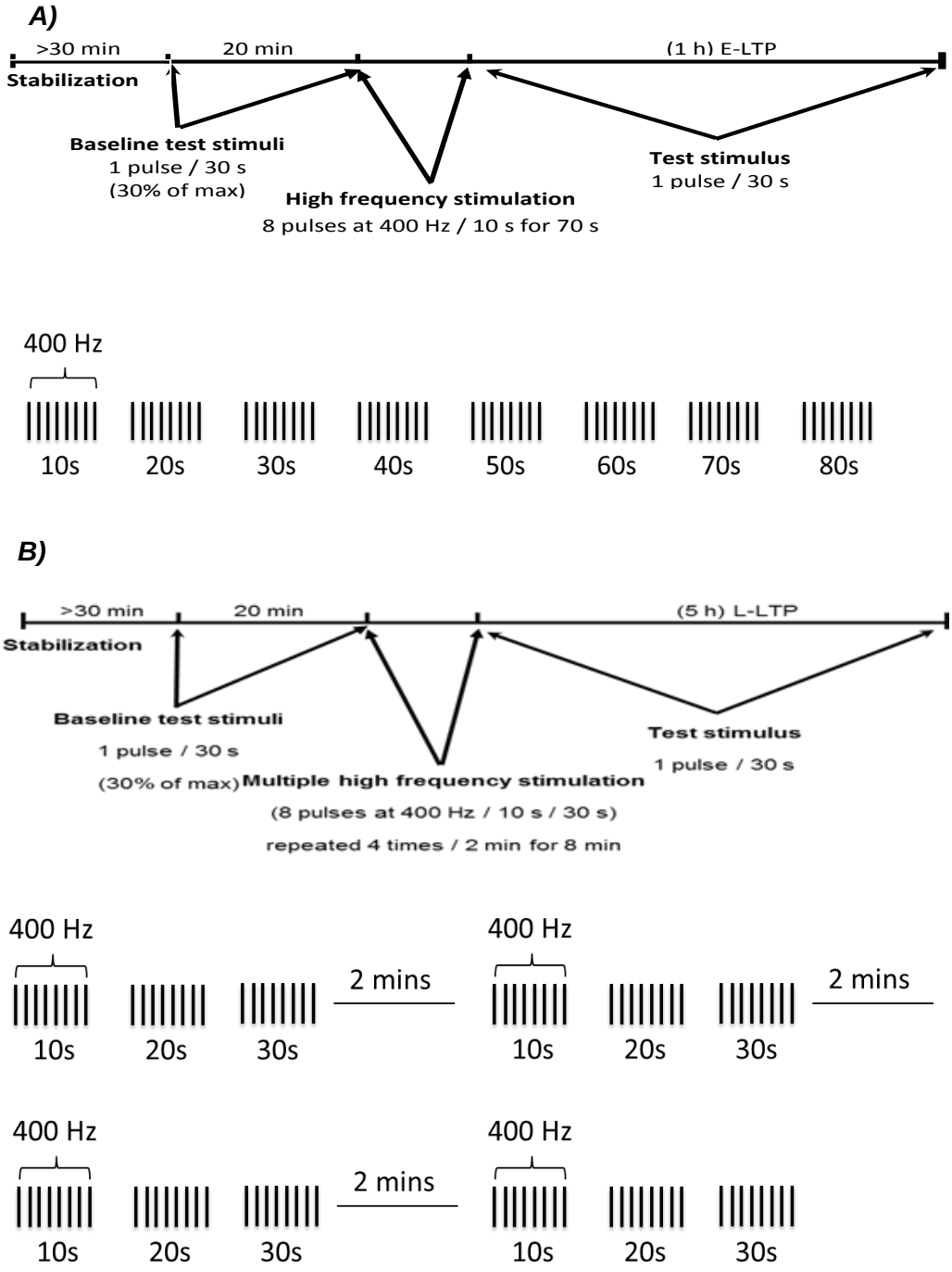

Figure 22. Steps for the induction of A) Early long-term potentiation (E-LTP) by HFS and B) Late long-term potentiation (L-LTP) by MHFS.

3.5. Western blotting:

3.5.1. Hippocampus dissection:

At the end of all treatments, rats were euthanized by giving a lethal injection of urethane into the heart. The brain dissection was performed for the basal, unstimulated (no LTP induction), and stimulated (after LTP induction) experiments. In basal dissection, the right hippocampus of each rat was grossly dissected and separated into the CA1 and DG sub-regions and these both areas from the same animal were kept for further analysis.

In unstimulated and stimulated dissections, the hippocampus sub-regions were dissected out similar in basal dissection but in a particular animal, only the sub-region being used for E-LTP or L-LTP study was dissected out. For example, dissection of the CA1 area was done after E-LTP or L-LTP recordings in pyramidal cells of CA1 area. Each sub-region was laid flat on a dry ice-filled Petri dish with the filter paper soaked with 0.2M sucrose and was positioned in such a way that the septal portion was on the left hand side and the temporal portion was on the right hand side. The two left and right tips were trimmed off. A small region in the middle of the hippocampus was removed to prevent overlapping of sub-regions. Then, the CA1 and DG sub-regions were further separated into septal and temporal portions and stored at -80^{0}C for later processing. The temporal tissue samples serve as internal controls because under stimulated conditions, the bulk of stimuli goes to the septal side while the temporal side receives negligible stimuli (Papatheodoropoulos and Kostopoulos, 2000).

3.5.1. Tissue homogenization and protein estimation:

Tissue was homogenized with 200 µL lysis buffer containing 50 mM Tris-HCl (pH=7.4), 1% NP-40, 0.1%SDS, 150 mM NaCl, 1mM EDTA, 1mM EGTA, 5 mM $Na_4P_2O_7$, 100 µg/ml PMSF, 40 mM β-glycerophosphate, 1 mM PMSF, and protease cocktail. Then, the samples were sonicated 3 times, 5 seconds/time (Vibra cell, Sonics and Materials Inc., Newtown, CT). A microBCA assay kit was used to estimate the amount of protein in each sample by constructing concentration curves from standard samples (Pierce Chemical Rockford, IL).

3.5.2. Immunoblotting and detection:

The samples (approximately 10-15 µg of total protein per sample) were processed via a high throughput system E-PAGE 48 (Invitrogen Corp. Carlsbad, CA). The proteins were transferred onto a PVDF membrane via a dry blot system (Invitrogen Corp. Carlsbad, CA) and detected with specific primary antibodies and subsequent conjugation with secondary horse-radish peroxidase antibodies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. Antibodies dilutions were done as details in Table 1. All antibodies were purchased from Santa Cruz Technology except p-CREB and GAPDH antibodies, which were purchased from Cell Signaling Inc., Boston, MA. The protein bands were visualized via commercial chemiluminescence reagents (Santa Cruz Biotechnology) and AlphaEase software and quantified by densitometry.

3.6. Statistical analysis:

Unpaired t-test was used to compare two groups while one-way analysis of variance (ANOVA) followed by Tukey post-hoc test was used to compare all groups. All statistical analyses were done with GraphPad Prism software. $P<0.05$ indicates statistical significance. Data were expressed as mean $\pm$ S.E.M.

4. RESULTS

4.1. Behavioral assessments:

Spatial learning and memory is generally known as hippocampus-dependent function. It is well-recognized that the hippocampus is the first critical region of the brain affected by Alzheimer's disease; thus leading to irreversible learning and memory impairment. This phenotype of AD pathology was successfully reproduced in several animal models; one such is the rat model of AD by i.c.v. infusion of amyloid peptides. Additionally, other symptoms of AD manifest as non-cognitive disturbances as the disease progresses in the human population. We are the first to report the anxiety-like behaviors in a rat model of AD. In this study, we reported that rats with 2 weeks of $A\beta_{1-42}$ infusion resulted in learning and memory deficits and anxiety-like behaviors while 4 weeks of treadmill exercise totally prevented these impairments.

4.1.1. Treadmill exercise prevented AD-induced learning and memory performance:

In the RAWM task, the number of errors made by rats in each learning trial or memory test was used as a quantitative measurement of learning and memory function. During the first six learning trials, rats in all groups made similar number of errors. However, during the second set of learning trials, the Aβ rats made significantly more errors than other groups (p= 0.01-0.05) (Figure 23A). In contrast, Ex/Aβ rats showed a learning ability similar to that of the control and exercised rats as these rat groups learn at equivalent rates. These results suggest that amyloid infusion impairs the learning ability in rats while regular treadmill exercise prevents this impairment.

In the STM test, administered 30 minutes after the last learning trial, the Aβ rats made significantly higher number of errors than all other groups (p= 0.01) (control: 0.5 ± 0.224, Ex: 0.917 ± 0.26, Aβ: 2.545 ± 0.529, Ex/Aβ: 0.917 ± 0.358) (Figure 23B). Four weeks of treadmill exercise reduced the number of errors the Aβ rats made, which was not significantly different from those of control and exercise rats. Same trend was observed in the LTM test, which was carried out 24 hrs after the 12^{th} learning trial. Aβ rats made an average of 3.75 ± 0.676 errors in the LTM task which was significantly higher compared to all other groups. The ability of Ex/Aβ rats to recall the RAWM task after 24 hrs was not different from those of control and exercise rats as the number of errors made in the LTM test were similar in these groups (control: 0.9 ± 0.348, Ex: 1.273 ± 0.524, Ex/Aβ: 1.6 ± 0.427) (Figure 23C). It is noteworthy that our exercise regimen in normal rats did not enhance performance as both the sedentary control and exercised rats performed similarly in this behavioral paradigm. Hence, at the behavioral level, regular exercise prevented the AD-induced learning and memory deficits.

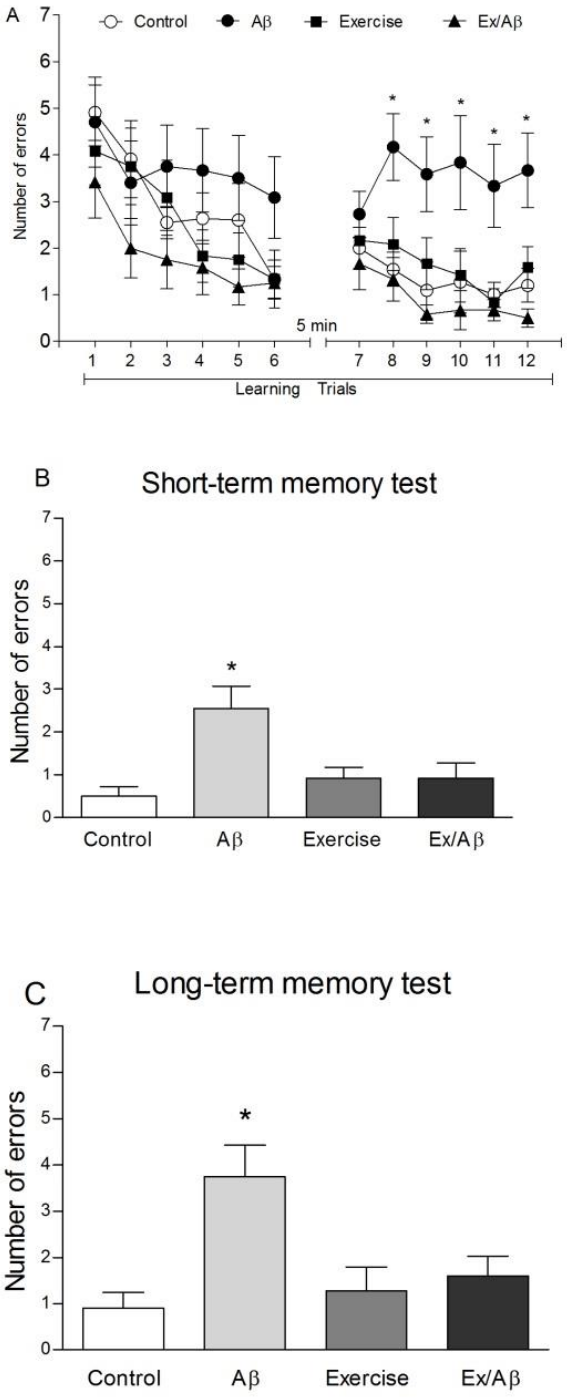

Figure 23. Radial arm water maze (RAWM) performance in sedentary or exercised rats with and without $A\beta_{42}$ infusion. The Aβ group reveals impairment in the learning trials (A), short-term memory test (B) and long-term memory test (C). Rats in the Aβ group make more errors compared to other groups during the last 5 learning trials and the memory tests. Ex/Aβ rats perform similarly as the control and exercise rats. Note that exercise rats show no significant difference from controls. (*) denotes significant difference from all groups ($p<0.05$, 10-12 rats/group).

4.1.2. Non-cognitive disturbances caused by AD pathology were totally prevented by regular treadmill exercise.

In the open field (OF) apparatus, the duration that the rat spent in the center area measures exploratory behaviors and anxiety levels (Gould et al., 2009, Salim et al., 2010). Time spent in the center area indicates high exploratory behaviors and low anxiety levels. In this study, the Aβ rats spent an average of 20.19 ± 4.428 percent time in the center area, which is significantly shorter than those of other groups (control: 40.87 ± 3.383%, exercise: 40.074 ± 5.409%, Ex/Aβ: 43.4 ± 4.612%, p = 0.01-0.05) (Figure 24A). Thus, it seems that regular exercise exerts an anxiolytic effect in our model of AD-like pathology. Also, in the OF apparatus the rat's locomotor activity is determined by total distance traveled and total activity. Aβ-infused rats traveled an average distance of 3371.974 ± 426.33 cm, which was similar to that of Ex/Aβ rats (3658.551 ± 155.279 cm), but statistically different from those of control and exercise rats (control: 5386.464 ± 309.939 cm, exercise: 5526.842 ± 348.041 cm, p= 0.001 - 0.01) (Figure 24B). Similar trend was observed with total activity in the OF where rats from Aβ and Ex/Aβ groups exhibited the same level of locomotor activity, but significantly different from those of control and exercise groups (p= 0.001 - 0.01) (Figure 24C). These data indicate that AD pathology impaired locomotor activity and this impairment was not prevented by our regimen of regular exercise.

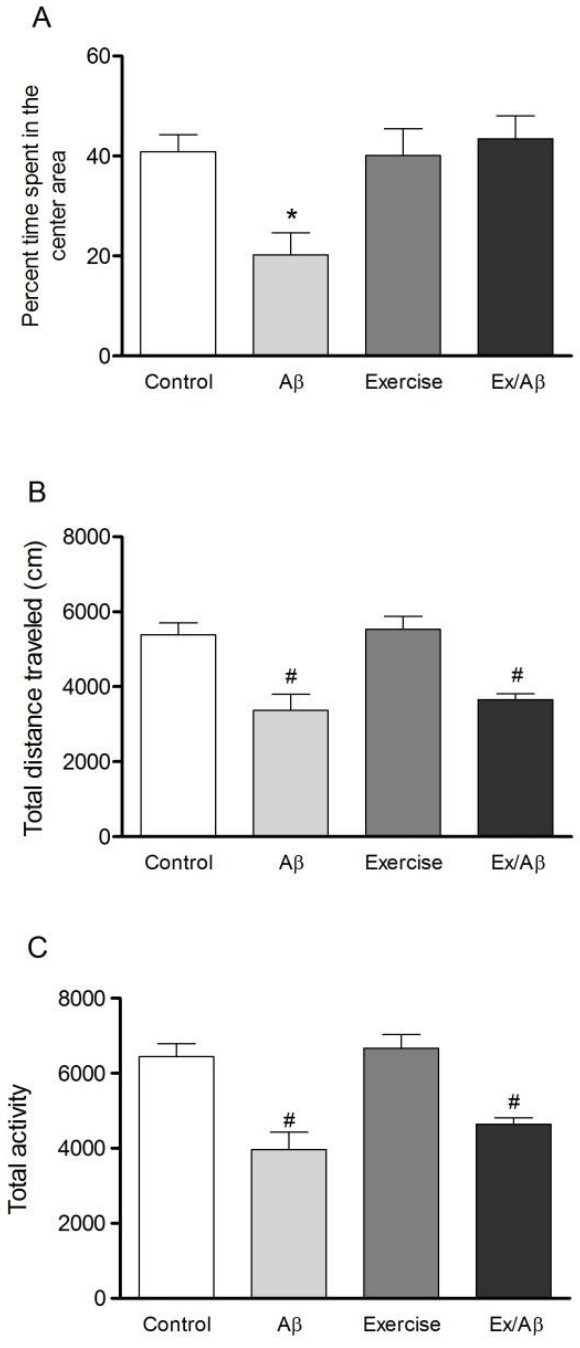

Figure 24. Alzheimer's disease (AD)-like pathology produces increased anxiety-like behavior, which is prevented by moderate treadmill exercise. Rats are placed in open field (OF) apparatus, a test of anxiety-like behavior. Aβ rats spend significantly less time in the center area in the OF test (A) compared to all groups while Ex/Aβ rats spend similar center time compared to control and exercise rats. The locomotor activity of both Aβ and Ex/Aβ rats are impaired as indicated by the reduced total distance traveled (B) and total activity (C) compared to control and exercise rats. (*) denotes significant difference from all groups, ($^{\#}$) indicates significant difference from control and exercise groups ($p<0.05$, 8-10 rats/group).

We have also utilized the advantage of light-dark box apparatus, which is a sensitive test of anxiety-like behavior. Because rodents are nocturnal, they have a tendency to stay in dark areas. Time spent in the light area indicates less anxiety-like behaviors (Salim et al., 2010, Vollert et al., 2011). We observed that control, exercise, and Ex/Aβ groups spent a similar length of time in the light compartment for exploring while Aβ rats spent a significantly less time in the light area (control: 121.74 ± 10.501 seconds, Aβ: 69.8 ± 10.671 seconds, exercise: 123.7 ± 8.179 seconds, Ex/Aβ: 108 ± 6.392 seconds, p = 0.01 - 0.05) (Figure 25). Hence, these results indicated that the anxiety-like behaviors induced by AD pathology could be alleviated by regular exercise.

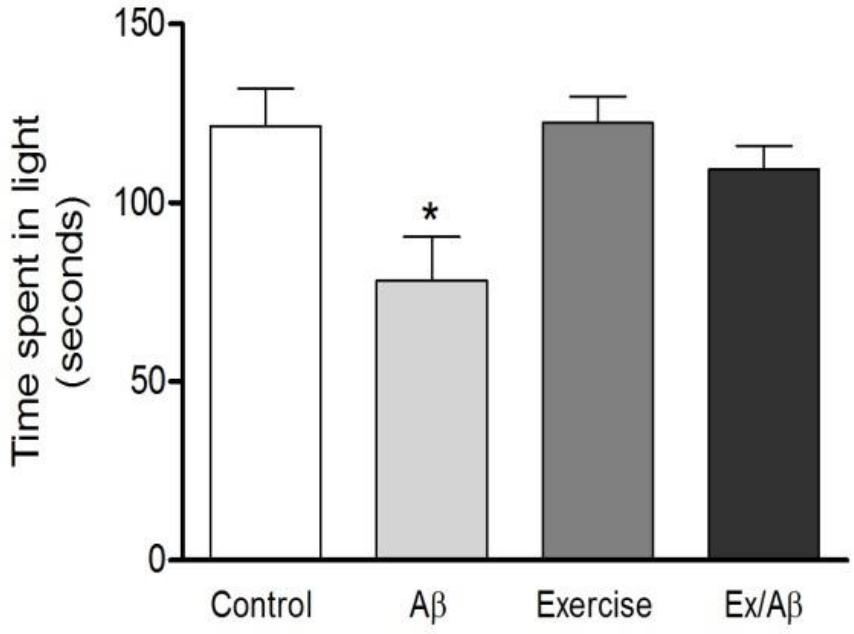

Figure 25. The effect of treadmill exercise on anxiety-like behavior of rats that have been infused with amyloid peptides. In the light-dark paradigm, Aβ rats show an increased anxiety-like behavior while Ex/Aβ rats seem to be normal as compared to control and exercise rats. (*) denotes significant difference from all groups ($p<0.05$, 8-10 rats/group).

The elevated plus maze (EPM) is used as a simple assay for assessing anxiety based on the natural aversion of rodents to elevated open areas (Xiang et al., 2011). Duration of time spent in the closed arms and center area measures anxiety levels. Consistent with our findings in the OF paradigm, AD rats experience increased anxiety-like behavior as these rats spent a significantly more time in the closed arms (control: 230.22 ± 13.621 seconds, Aβ: 279.778 ± 4.325 seconds, exercise: 225.374 ± 19.487 seconds, Ex/Aβ: 250.363 ± 10.179 seconds, p= 0.05 - 0.01) (Figure 26A) but much less time in the center area (control: 56.245 ± 10.691 seconds, Aβ: 13.417 ± 3.124 seconds, exercise: 45.071 ± 9.974 seconds, Ex/Aβ: 37.904 ± 8.215, p= 0.002 - 0.03) (Figure 26B) compared to all other groups. Additionally, similar to what was observed in the OF, the EPM data showed that the locomotor activity of Aβ-infused rats was impaired and our exercise regimen did not prevent this impairment as the Aβ and Ex/Aβ rats traveled similar distance during the whole experiment (Aβ: 1152.229 ± 134.25 cm, Ex/Aβ: 1260.222 ± 71.279 cm) but significantly different than those of the control and exercise rats (control: 1662.167 ± 84.193 cm, exercise: 1528.571 ± 48.672 cm, p= 0.01 - 0.03) (Figure 26C).

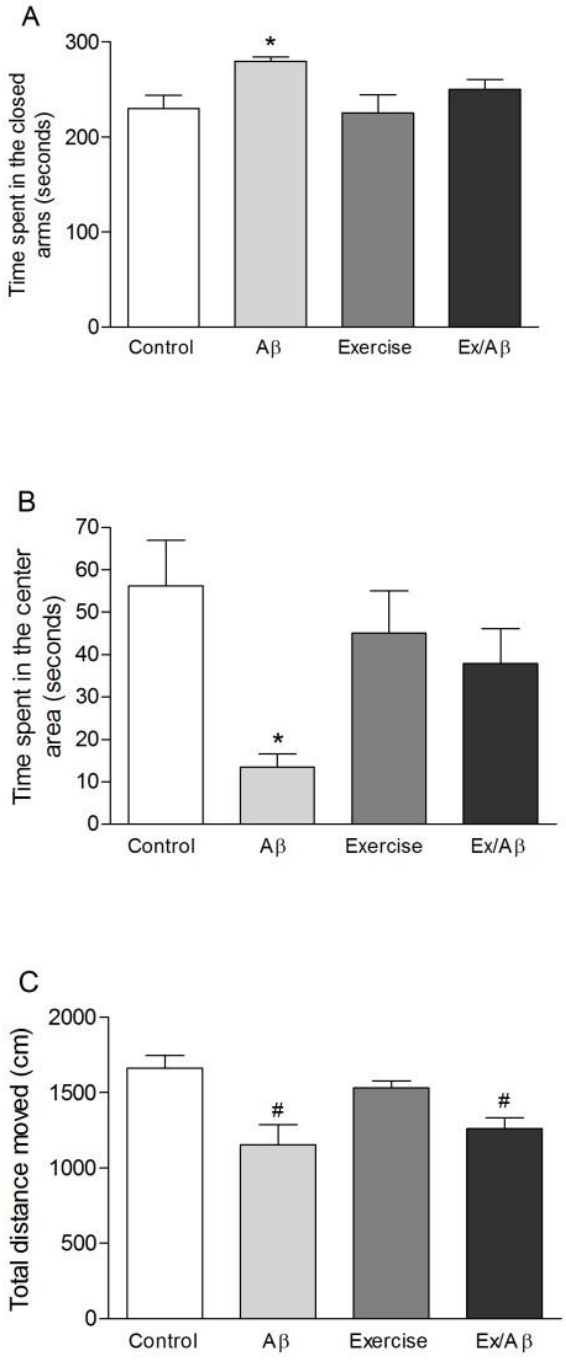

Figure 26. Elevated plus maze (EPM) exploration in rats treated with $A\beta_{1-42}$ peptides and/or exercise training. (A): Time spent in the closed arms, (B): Time spent in the center area, (C): Total distance travelled. Aβ rats show increased anxiety-like behaviors as indicated by significantly longer time in the closed arms and shorter time in the center area compared to all other groups and while this aberrant behavior is absent in Ex/Aβ rats. The impaired locomotor activity of Aβ rats (shorter distance traveled) is not prevented by treadmill exercise. (*) $p<0.05$ compared to all groups, ($^{\#}$) $p<0.05$ compared to control and exercise groups, 8-10 rats/group.

4.2. Electrophysiological recordings:

It is well-established that both *in vivo* and *in vitro* accumulation of toxic amyloid peptides interferes with synaptic transmission of the excitatory glutamatergic system (Brouillette et al., 2012, Doherty et al., 2013). We have utilized the advantages of extracellular recordings in live animals to assess the changes at the synapses caused by continuous $A\beta_{1-42}$ infusion and/or chronic moderate treadmill exercise training in both CA1 and DG sub-regions of the hippocampus.

4.2.1. Basal synaptic transmission:

We have evaluated the effect of AD pathology and/or exercise on basal synaptic transmission in both CA1 and DG sub-regions by constructing input-output (I/O) curves. I/O curves are used as an index of synaptic strength as the postsynaptic cell respond differently to various stimulus intensities. The I/O curve will change when synaptic strength is altered (Alzoubi et al., 2010, Alzoubi et al., 2011). Our data indicated that Aβ rats exhibited impaired basal synaptic transmissions in both sub-regions of the hippocampus.

In CA1 area, the I/O curves of Aβ rats was shifted to the right with a significantly lower fEPSP slope at all stimulus intensities compared to all other groups ($p = 0.01$) (Figure 27A). However, there were no changes observed between the I/O curves of control, exercise, Ex/Aβ rats. For instance, at minimal intensity, the fEPSP slope of Aβ rats was 0.089 ± 0.012 mV/ms which is markedly lower compared to all groups (control: 0.275 ± 0.032 mV/ms, Ex: 0.285 ± 0.047 mV/ms, Ex/Aβ: 0.235 ± 0.037 mV/ms).

Even though the granule cells of the DG area tend to produce a more robust post-synaptic response compared to the pyramidal CA1 cells, similar trend was observed in the DG area. For example, Aβ rats exhibited a significant lower fEPSP slope at all stimulus intensities compared to those of control, exercise and Ex/Aβ rats (p= 0.001) (Figure 28A). For example, at minimal intensity, the fEPSP slope of Aβ rats was 2.585 ± 0.286 mV/ms which was much smaller than those of control (4.62 ± 0.323 mV/ms), exercise (3.979 ± 0.04 mV/ms), and Ex/Aβ (4.215 ± 0.359 mV/ms) rats. Thus, it seems that AD pathology severely impairs basal transmissions of the perforant and Schaffer collaterals synapses while regular moderate treadmill exercise protects these synapses.

In addition to I/O curves, basal synaptic transmissions in both CA1 and DG sub-regions were further assessed by the magnitude of the voltage that required to elicit the minimal, 30% of maximal and maximal responses. As demonstrated in Figure 27B, Aβ rats required a significant higher voltage to produce the same response in CA1 area with control, exercise, and Ex/Aβ rats at minimal, 30% of maximum and maximal (p= 0.001 - 0.01). In particular, a mean voltage of 6.767 ± 0.297 mV was required to potentiate minimal response in Aβ rats while Ex/Aβ rats could produce the same response at 5.064 ± 0.166 mV which was not different from those of control (5.08 ± 0.343 mV) and exercise (5.157 ± 0.441 mV) rats. Similar trend was observed in the DG area. For example, Aβ rats required a mean voltage of 6.26 ± 0.189 mV to elicit a minimal response at the perforant synapses which was markedly higher compared to those of all other groups (control: 4.475 ± 0.382 mV, exercise: 3.58 ± 0.18 mV, Ex/Aβ: 5.14 ± 0.299

mV) (Figure 28B). Altogether, these data suggested that the impaired basal synaptic transmission caused by AD pathology was prevented by prior treadmill exercise.

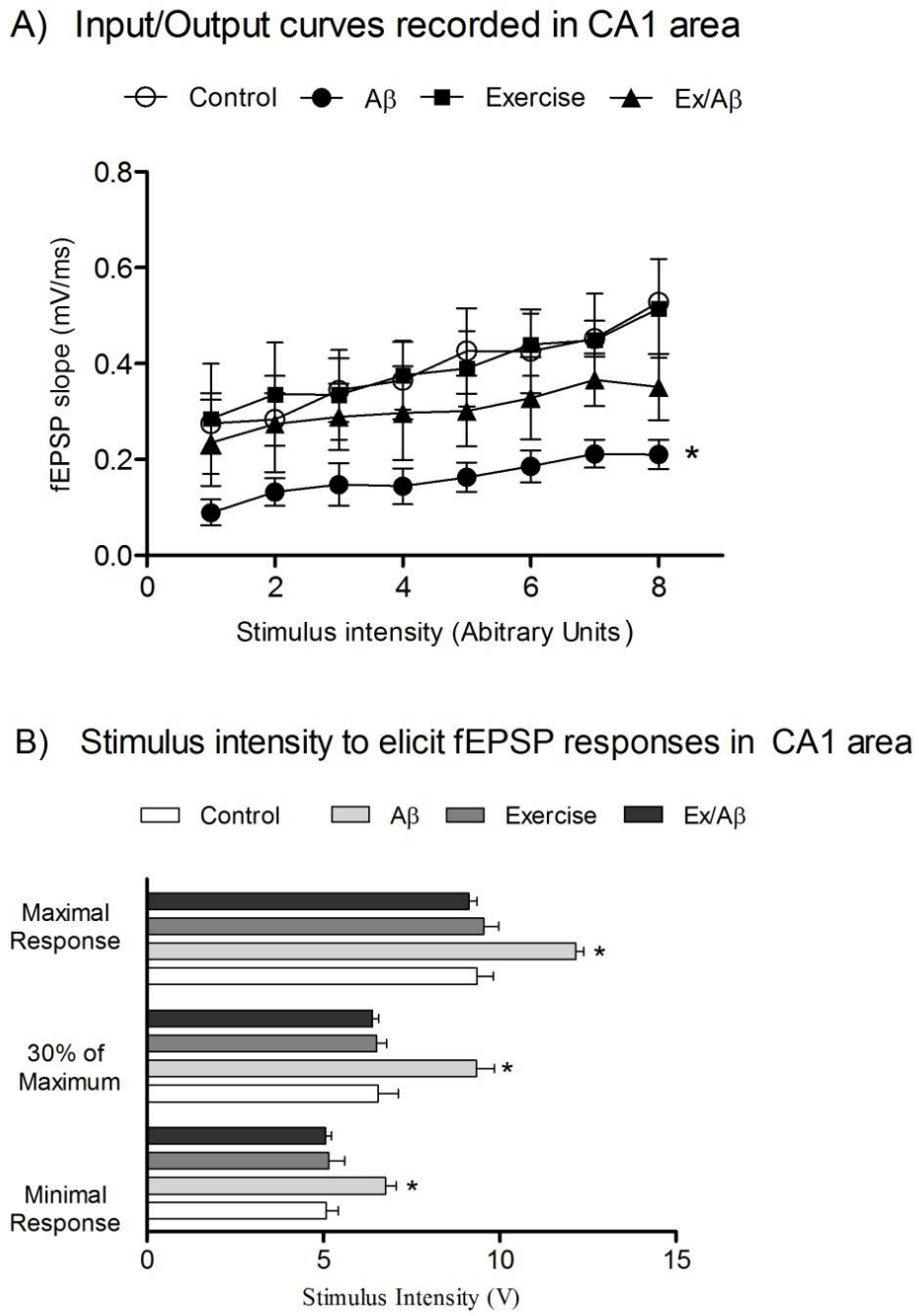

Figure 27. Basal synaptic transmission is impaired in CA1 area of Aβ rats and this impairment is prevented by moderate treadmill exercise. (A): the input-output (I/O) curves are indices of synaptic responses (i.e. field excitatory post-synaptic potential (fEPSP) slope) constructed from gradual increases in stimulus intensity. (B): Stimulus intensity required to produce minimal, 30% of maximum, and maximal response. The right shift of the I/O curve of Aβ rats indicates impaired basal synaptic transmission while this shift is not seen with the I/O curve of Ex/ Aβ rats. Aβ rats also require a significantly stronger voltage to elicit the same response compared to control, exercise, and Ex/Aβ rats. (*) indicates significant difference compared to other groups at all stimulus intensities. Values are mean ± S.E.M., n = 4-6 rats/group.

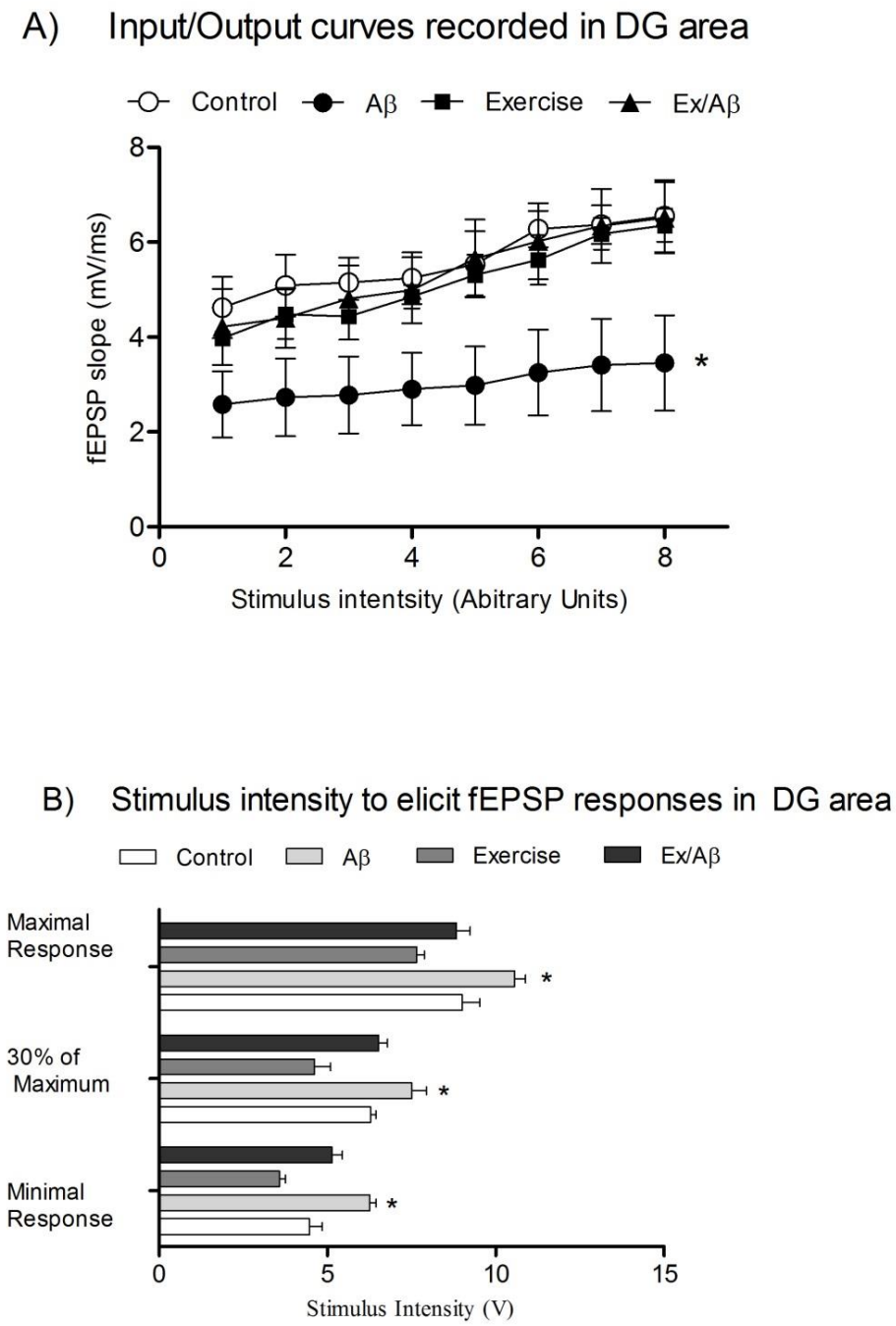

Figure 28. AD pathology impairs basal synaptic transmission in the DG area of Aβ rats while moderate treadmill exercise prevents this impairment. (A): the input-output (I/O) curves are indices of synaptic responses (i.e. field excitatory post-synaptic potential (fEPSP) slope) constructed from gradual increases in stimulus intensity. (B): Stimulus intensity required to elicit minimal, 30% of maximum, and maximal response. The I/O curve of Aβ rats is altered while this of Ex/Aβ rats is not different from controls. Aβ rats also require a significantly stronger voltage to elicit the same response compared to control, exercise, and Ex/Aβ rats. (*) indicates significant difference compared to other groups at all stimulus intensities. Values are mean ± S.E.M., n = 4-6 rats/group.

4.2.2. Synaptic transmissions after repetitive high frequency stimulation:

Early and late phase long-term potentiation (E- and L-LTP) are considered to be the closest cellular analogues of short-term and long-term memory respectively. Evidence from our labs and others reported E-LTP and L-LTP inhibition in various learning and memory deficits models including sleep deprivation, maternal deprivation, and Alzheimer's disease (Alkadhi et al., 2011, Zagaar et al., 2012, Dao et al., 2013, Marco et al., 2013, Zagaar et al., 2013). Thus, in this study we evaluated the effects of 4 weeks of treadmill exercise on repetitive high frequency stimulation (HFS)- evoked E-LTP and L-LTP magnitudes in both CA1 and DG areas of the hippocampus.

4.2.2.1. Regular treadmill exercise prevented AD-induced suppression of early phase long-term potentiation (E-LTP) in the hippocampus

Compared to baseline, the fEPSP slope from the Aβ rat group in CA1 area was significantly lower than that of other groups (p= 0.001-0.05) (control: 141.29% ± 8.24, Aβ: 106.93% ± 2.99, Ex: 138.36% ± 3.5, Ex/Aβ: 140.7% ± 7.36) (Figure 29A). This result indicates an impaired synaptic plasticity in this AD rat model. Interestingly, one hour after HFS, the fEPSP slope of Aβ rats was back to the baseline value. In contrast, the fEPSP slope of Ex/Aβ rats was similar to that of control rats throughout the recording period of one hour. Thus, treadmill exercise prevented AD-induced synaptic plasticity inhibition in Aβ rats. Additionally, 1 hour after HFS the pspike amplitude of Aβ rats (92.81% ± 7.99) was markedly lower than other groups (p= 0.001-0.05) (control: 184.94% ± 8.79, Ex: 168.79% ± 10.35, Ex/Aβ: 132.98% ± 7.03) (Figure 29B).

Similar trend was observed in the granule cell layer recording of the DG area. Single train of high frequency stimulation induced a robust post-synaptic response of the perforant synapses in all groups except the Aβ group. In particular, 1 hr after HFS the fEPSP slope of Aβ rats returned to baseline (99.618% ± 7.417), which was significantly different from all groups (p= 0.001). The fEPSP slope of Ex/Aβ rats was 138.434% ± 9.136, which was similar to those of control (142.044% ± 4.835) and exercise (135.606% ± 6.569) rats (Figure 30A). Surprisingly, our regimens of amyloid infusion and/or exercise training did not alter the number of neurons that reach threshold and fire in the DG area as the pspike amplitudes were similar across all groups (control: 225.786% ± 14.311, Aβ: 254.697% ± 34.631, exercise: 231.245% ± 29.616, Ex/Aβ: 232.962% ± 40.488) (Figure 30B).

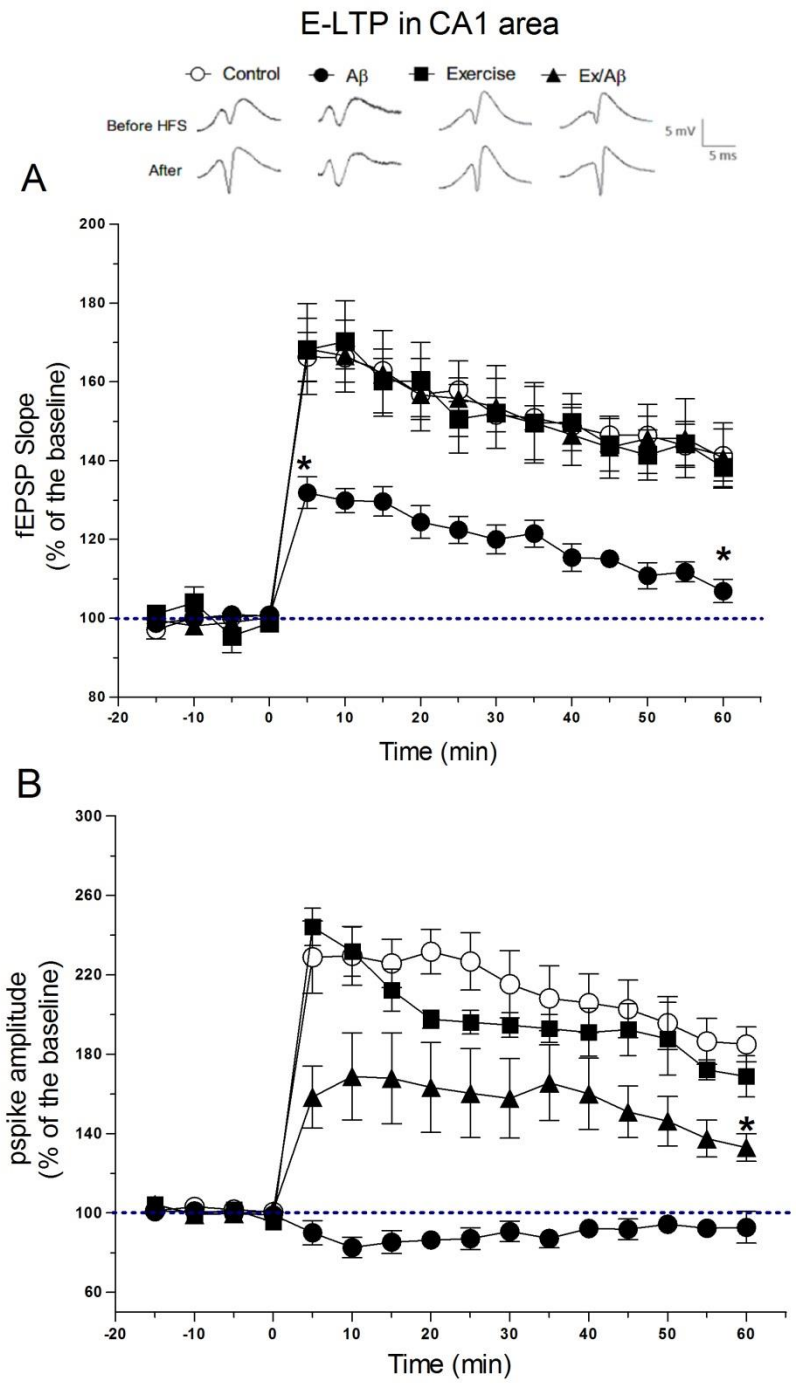

Figure 29. Hippocampal early phase LTP (E-LTP) is measured as increases in the slope of the fEPSP (A) and pspike amplitude (B) in CA1 area, which is evoked by HFS (applied at time zero) of the Schaffer collateral synapses in anesthetized rats. In rats with $A\beta_{1\text{-}42}$ infusion (Aβ), E- LTP is significantly more impaired than other groups. Ex/Aβ rats exhibits a similar fEPSP slope compared to that of control and exercised rats. Additionally, the pspike amplitude of Ex/Aβ rats is markedly different from those of Aβ rats. Each point is the mean ± SEM of 5-6 rats. Points between the two asterisks (*) indicate significant difference from all groups.

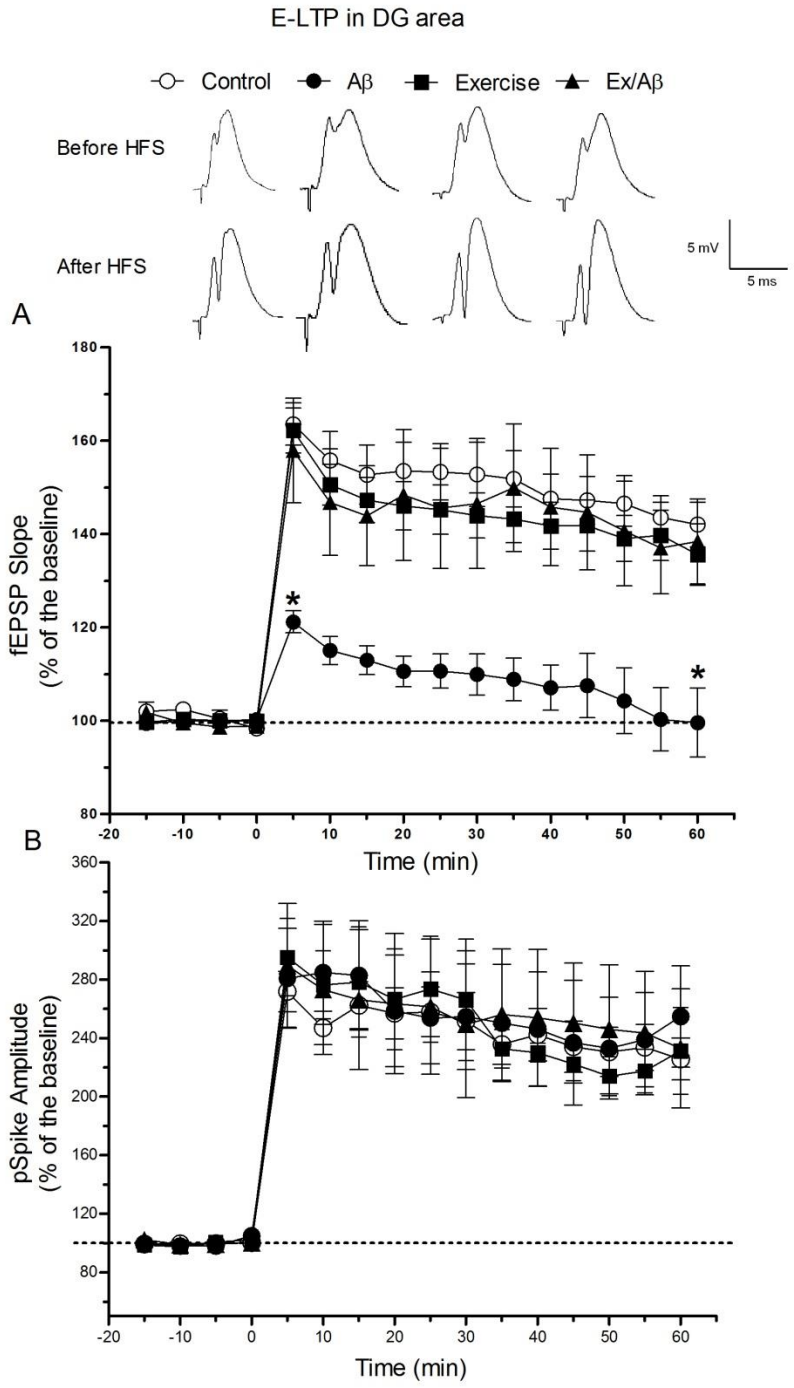

Figure 30. Early phase LTP (E-LTP) is measured in the DG area of the hippocampus: fEPSP slope (A) and pspike amplitude (B). Single train of HFS (applied at time zero) evokes E-LTP of perforant synapses in anesthetized rats. The fEPSP slope of Aβ rats is significantly lower compared to other groups while Ex/Aβ rats exhibits a similar fEPSP slope compared to that of control and exercised rats. Surprisingly, the pspike amplitudes of all rats including rats with amyloid peptides infusion alone are the same. Each point is the mean ± SEM of 5-6 rats. Points between the two asterisks (*) indicate significant difference from all groups.

4.2.2.2. Late phase long-term potentiation (L-LTP) impairment induced by AD pathology was prevented by moderate treadmill exercise

Next, we investigated the effects of toxic amyloid infusion and/or exercise training on the synapses of the CA1 and DG areas. L-LTP was induced by multiple trains of HFS and recorded for 5 hours. The fEPSP slope of Schaffer collaterals pathway in the CA1 area remained similar throughout the 5 hrs recording period among control, exercise, and Ex/Aβ rats (control: 155.797% ± 17.652, exercise: 139.654% ± 9.874, Ex/Aβ: 145.511% ± 5.799) (Figure 31A). However, Aβ rats exhibited a markedly lower fEPSP slope in CA1 area from the beginning of induction of L-LTP to the end of the recording.

Even though the pspike amplitude of rats that have been infused with amyloid peptides and exercised was not comparable to those of control and exercise rats, it was significantly different from that of Aβ rats. For example, compared to baseline right after L-LTP induction the pspike amplitude of Aβ rats was 138.483% ± 12.926 and drastically dropped even below baseline (91.108% ± 6.671) after 5 hrs. Meanwhile, the pspike amplitudes of control, exercise, and Ex/Aβ rats were 210.394% ± 25.608, 229.981% ± 12.365, 139.497% ± 19.566 respectively at the end of the recording period (Figure 31B).

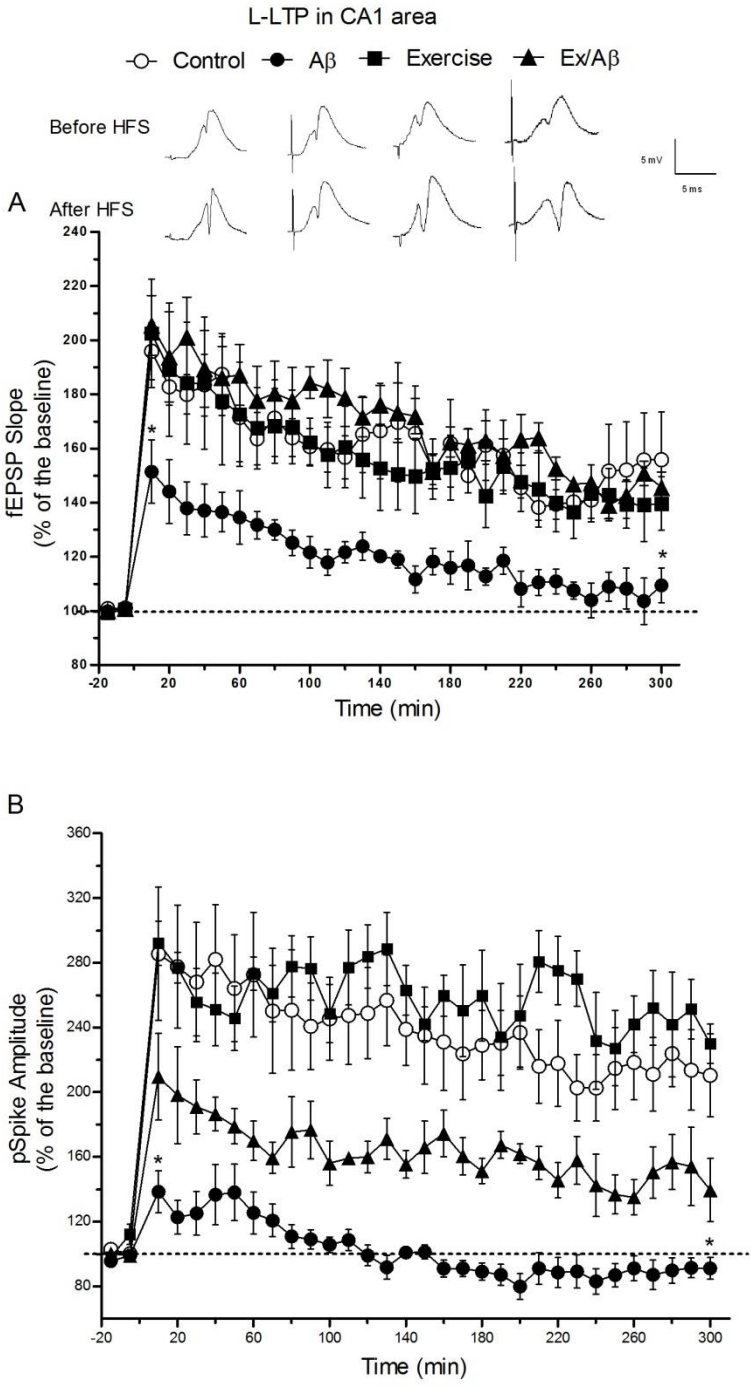

Figure 31. Hippocampal late phase LTP (L-LTP) is measured as increases in the slope of the fEPSP (A) and pspike amplitude (B) in CA1 area, which is evoked by multiple trains of HFS (applied at time zero) of the Schaffer collateral synapses in urethane-anesthetized rats. $A\beta_{1-42}$ infusion (Aβ) significantly impairs L-LTP as Aβ rats show a markedly lower fEPSP slope compared to other groups. Ex/Aβ rats exhibits a similar fEPSP slope compared to that of control and exercised rats. Additionally, the pspike amplitude of Ex/Aβ rats is markedly different from those of Aβ rats. Each point is the mean ± SEM of 5-6 rats. Points between the two asterisks (*) indicate significant difference from all groups.

Similarly, granule cells of DG area failed to produce a robust fEPSP slope upon multiple trains of HFS in Aβ rats and this deficit was totally prevented by exercise training. The fEPSP slope in DG area of Ex/Aβ rats was 156.522% ± 7.532 which was not significantly different from those of control (137.68% ± 8.341) and exercise (138.061% ± 13.713) rats but markedly higher than that of Aβ rats (97.159% ± 7.00) (p= 0.001) (Figure 32A). To our surprise, it seemed that the number of granule cells that reached threshold and fired were unaffected by AD pathology and/or exercise training. There were no significant differences in the pspike amplitude in DG area upon L-LTP induction among all groups (control: 217.372% ± 11.076, exercise: 225.416% ± 28.83, Aβ: 219.13 ± 38.983, Ex/Aβ: 195.51% ± 12.162) (Figure 32B).

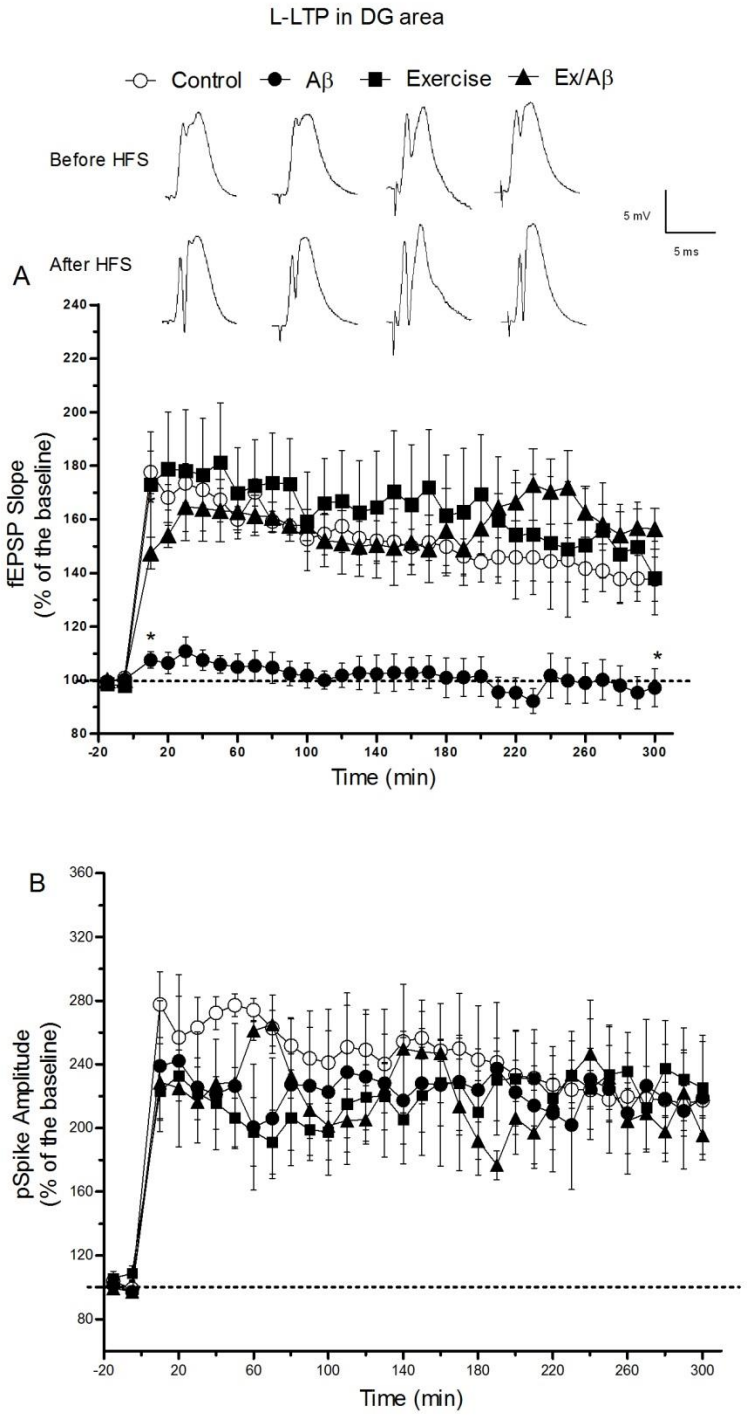

Figure 32. Late phase LTP (L-LTP) is measured in the granule cell layer of the hippocampal DG area: fEPSP slope (A) and pspike amplitude (B). L-LTP of the perforant synapses is induced by multiple trains of HFS in urethane-anesthetized rats. The fEPSP slope of Aβ rats is significantly lower compared to other groups while Ex/Aβ rats exhibits a similar fEPSP slope compared to that of control and exercised rats. However, the pspike amplitudes of all groups are not different. Each point is the mean ± SEM of 5-6 rats. Points between the two asterisks (*) indicate significant difference from all groups.

4.3. Molecular analysis:

4.3.1. Basal levels of molecules implicated in AD pathology in CA1 and DG areas

4.1.1. Basal levels of APP

Amyloid precursor protein (APP) can be processed via a family of secretases to produce amyloid peptides that can be toxic (e.g. $A\beta_{1-42}$). In this section, we examined the basal levels of APP in both CA1 and DG areas of rats that have been infused with amyloid proteins and/or exercise training. The level of APP in CA1 area of Aβ rats (1.469 ± 0.133) was significantly higher than the other groups (p= 0.05) while this level in Ex/Aβ rats (0.836 ± 0.108) was similar to those of control (0.979 ± 0.041) and exercise (0.923 ± 0.134) rats (Figure 33).

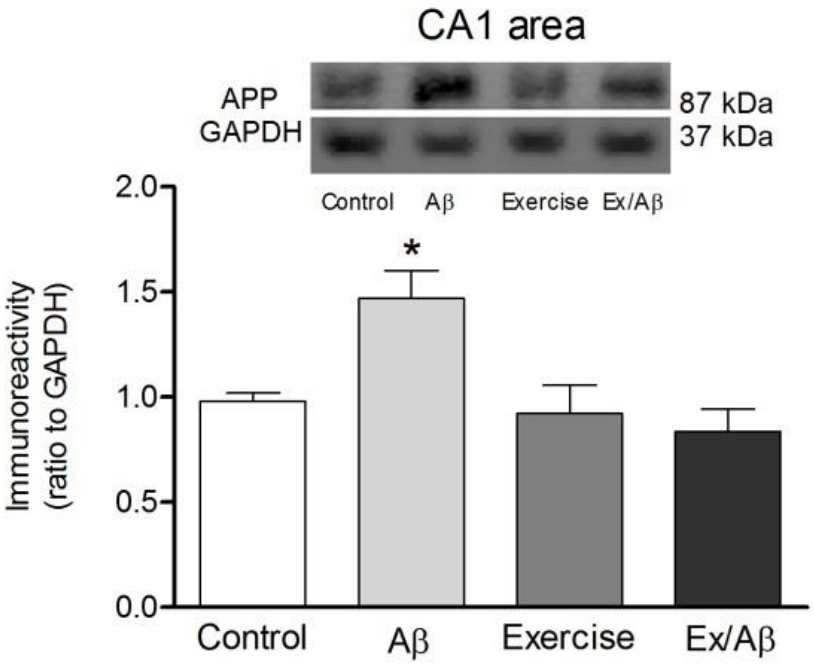

Figure 33. Basal levels of APP in CA1 area. In our rat model of Alzheimer's disease, the basal level of APP is highly elevated and this abnormal elevation is prevented by 4 weeks of treadmill exercise. The basal level of APP in Ex/Aβ rats is similar to those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

The effect of exercise on AD pathology in DG area was similar to that observed in area CA1. For example, Aβ rats had an average basal level of APP of 1.078 ± 0.275 which was markedly higher than all groups (p= 0.01 - 0.05). The basal level of APP in Ex/Aβ rats was only 0.6011 ± 0.1049 and those of control and exercise rats were 0.6948 ± 0.0491 and 0.6144 ± 0.1679 respectively (Figure 34). Thus, in our model, there was an increase in APP in both CA1 and DG sub-regions and this effect was prevented by moderate treadmill exercise.

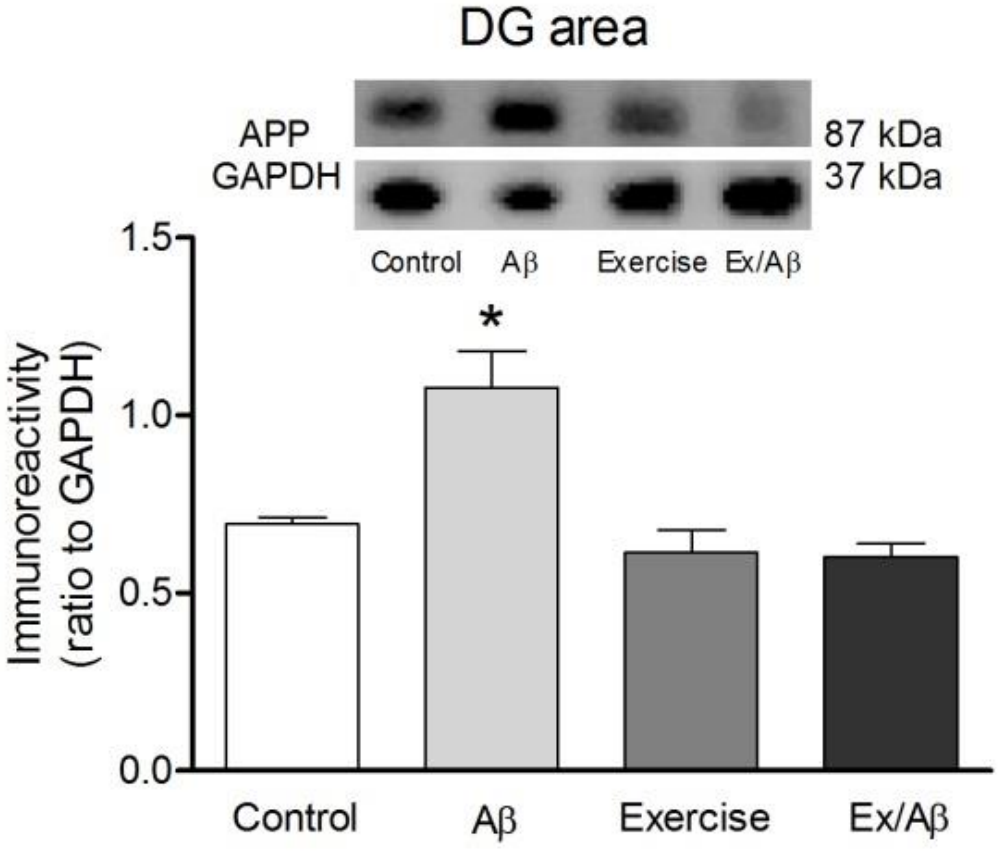

Figure 34. Basal levels of APP in DG area. Continuous amyloid infusion increases the basal level of APP while prior regular exercise removes this deleterious alteration. The basal level of APP in Ex/Aβ rats is similar to those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.1.2. Basal levels of BACE-1

The amyloidogenic pathway involves the sequential cleavage of APP by beta-site APP cleaving enzyme-1 (BACE-1). It has been shown that overproduction of amyloid products by BACE gene manipulation in mice leads to the increased propensity for amyloid aggregation (Vassar et al., 1999). In our study, western blots analysis of the CA1 area homogenates indicated that the basal level of BACE-1 was highly elevated in Aβ rats (1.462 ± 0.3673) and this elevation was absent in Ex/Aβ rats (0.8685 ± 0.1532), which was similar to those of control (0.9188 ± 0.1525) and exercise (0.9912 ± 0.1021) rats (p=0.05) (Figure 35).

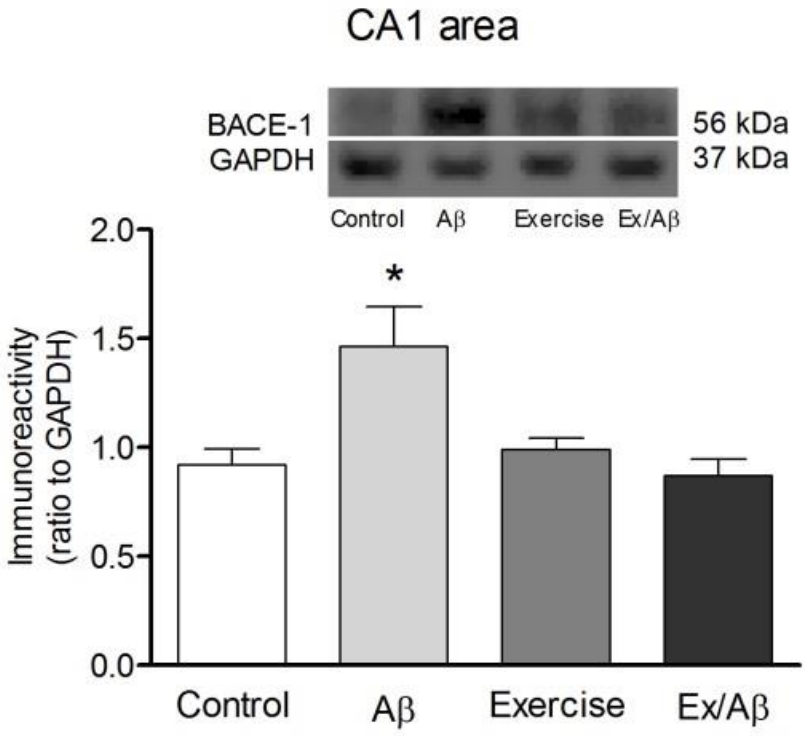

Figure 35. Immunoblot analysis of the basal levels of BACE-1 in the CA1 area. Continuous amyloid infusion increases the basal level of BACE-1 and this increase is prevented by treadmill exercise. The basal level of BACE-1 in Ex/Aβ rats is normal compared to those of control and exercise rats while this level is significantly high in Aβ rats. (*) indicates significant difference from control, exercise, and Ex/Aβ group ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

Similar trend was observed in the DG area as exercise training prevented the BACE-1 increase induced by AD pathology. The basal level of BACE-1 in DG area of Ex/Aβ rats was 0.9968 ± 0.1929, which was not different from those of control (0.9597 ± 0.2772) and exercise (1.004 ± 0.1018) rats but significantly reduced compared to that of Aβ rats (1.649 ± 0.5437) (p= 0.01) (Figure 36).

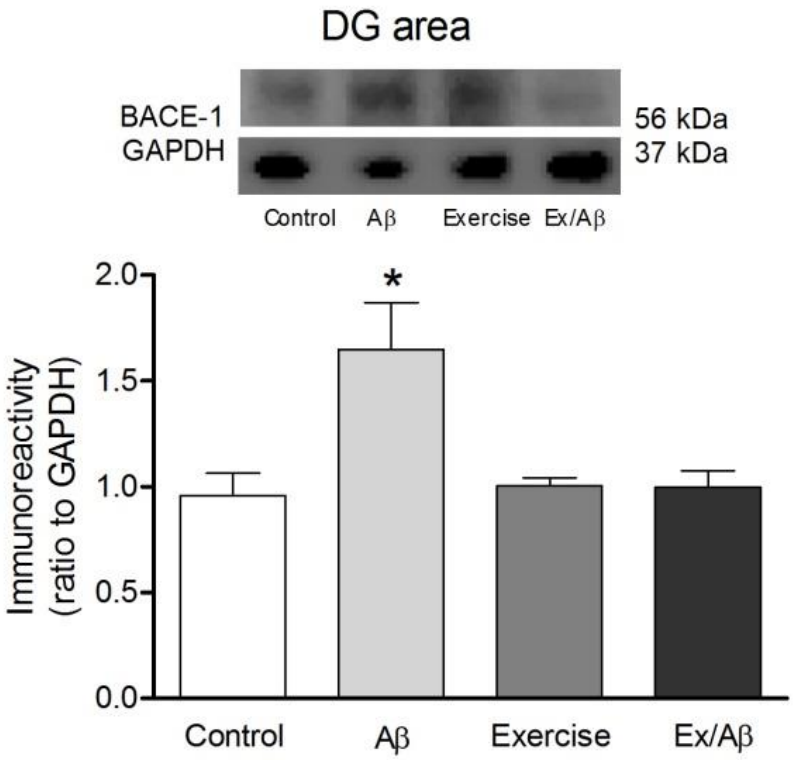

Figure 36. Immunoblot analysis of the basal levels of BACE-1 in total homogenates of the DG area. The basal level of BACE-1 is highly up-regulated in our AD model and this aberrant upregulation is normalized by prior treadmill exercise. The basal level of BACE-1 in Aβ rats is significantly higher compared to all groups while this level in Ex/Aβ rats is not different from those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2. Basal levels of molecules implicated in learning and memory and long-term potentiation

4.3.2.1. Basal levels of phosphorylated and total CaMKII

Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) is a memory molecule that has been intensively studied as a key to unlock cognition mysteries (Cammarota et al., 2002). Nevertheless, there has not been much research investigating the relationship between CaMKII and non-cognitive function. One recent study showed that mice with gap junction deficiency displayed enhanced anxiety-related behavior, which coincided with a reduction of CaMKII level in the striatum (Zlomuzica et al., 2012). Our data revealed that the basal levels of phosphorylated (p-) CaMKII, an active form of CaMKII in CA1 area was significantly reduced compared to other groups (control: 1.136 ± 0.081, Aβ: 0.293 ± 0.065, exercise: 1.439 ± 0.354, Ex/Aβ: 1.112 ± 0.137, p= 0.01 - 0.05) (Figure 37A). Interestingly, the basal levels of total (t-) CaMKII remained unchanged across all groups in CA1 area (control: 3.52 ± 0.483, Aβ: 3.859 ± 0.53, exercise: 3.576 ± 0.529, Ex/Aβ: 3.887 ± 0.297) (Figure 37B). As a result, the ratio of p-CaMKII to t-CaMKII of Aβ rats is significantly lower than those of control, exercise, and Ex/Aβ rats (control: 0.437 ± 0.062, Aβ: 0.148 ± 0.036, exercise: 0.475 ± 0.102, Ex/Aβ: 0.351 ± 0.057, p= 0.05) (Figure 37C). Thus, it seems that AD pathology primarily targets CaMKII phosphorylation but does not affect the synthesis of CaMKII while regular exercise prevents this functional phosphorylation impairment.

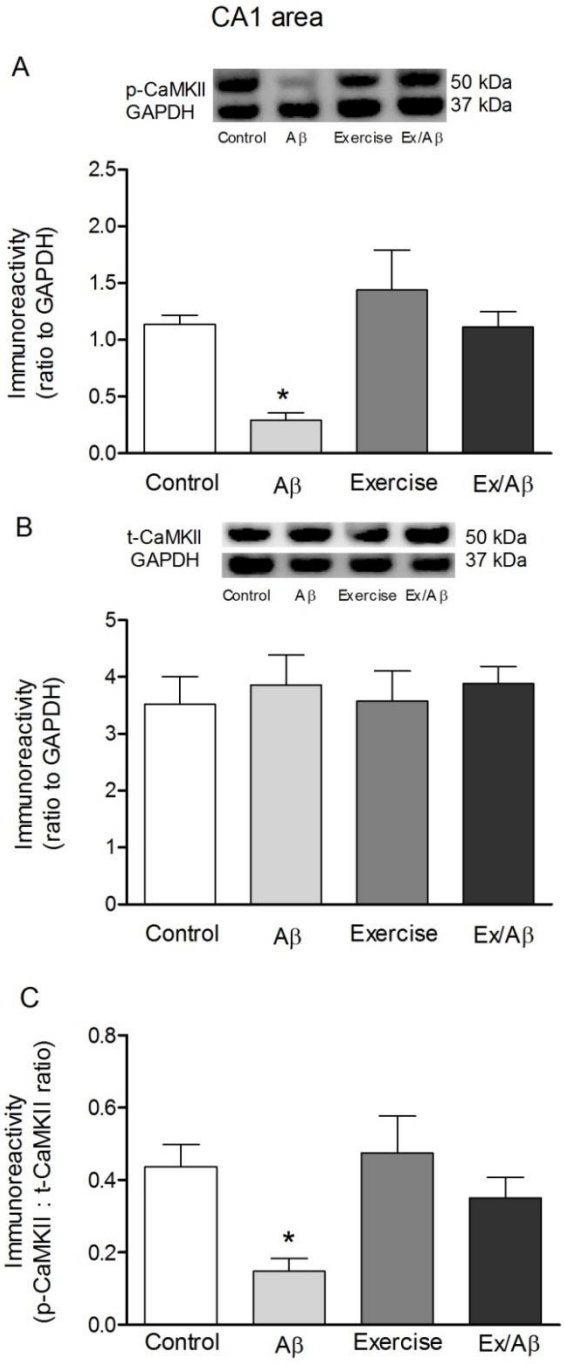

Figure 37. Basal levels of p-CaMKII (A), t-CaMKII (B), and p-CaMKII : t-CaMKII ratio (C) in CA1 area. The basal levels of p-CaMKII in Aβ rats are significantly reduced compared to other groups while the levels of t-CaMKII were similar across all groups. Thus, the p-CaMKII : t-CaMKII ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats, which indicates an impaired phosphorylation process. Ex/Aβ rats exhibits normal p-CaMKII basal levels and p- CaMKII : t-CaMKII ratio as compared to those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

Similar findings were observed in the DG area. In this hippocampal sub-region, the basal level of p-CaMKII in Aβ rats was 0.291 ± 0.068, which was much lower than those of control (1.0 ± 0.063), exercise (0.75 ± 0.083), and Ex/Aβ (1.021 ± 0.116) rats (p= 0.05-0.01) (Figure 38A). Nevertheless, the total pool of basal CaMKII in DG area were the same across all groups (control: 3.546 ± 0.414, Aβ: 3.14 ± 0.496, exercise: 2.92 ± 0.294, Ex/Aβ: 3.243 ± 0.448) (Figure 38B). Hence, the p-CaMKII : t-CaMKII ratio of Aβ rats (0.083 ± 0.015) was much smaller compared to control rats (0.302 ± 0.047) while this ratio of Ex/Aβ rats (0.339 ± 0.041) was similar to that of control and exercise rats (0.281 ± 0.032) was similar to those of control and exercise rats (p= 0.01 - 0.05) (Figure 38C).

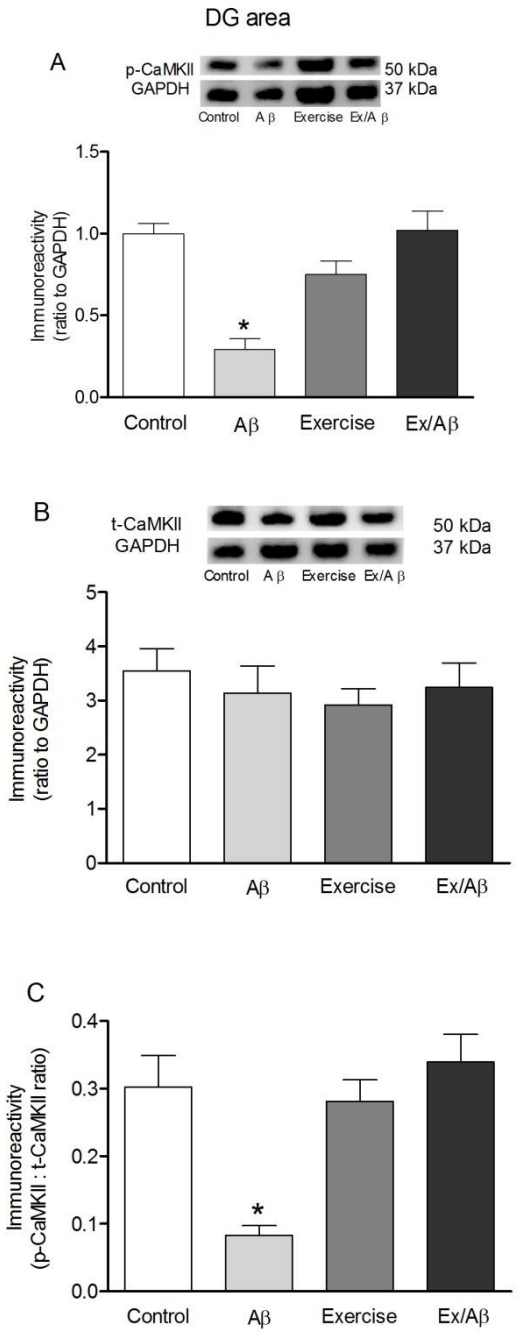

Figure 38. Basal levels of p-CaMKII (A), t-CaMKII (B), and p-CaMKII : t-CaMKII ratio (C) in DG area. The basal levels of p-CaMKII in Aβ rats are significantly decreased compared to other groups while the levels of t-CaMKII are similar across all groups. Thus, the p-CaMKII : t-CaMKII ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats, which indicates that AD pathology targeted primarily the phosphorylation process. Regular treadmill exercise prevents the reduction in p-CaMKII basal levels and p-CaMKII : t-CaMKII ratio of Aβ rats. (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2.2. Basal levels of calcineurin (PP2B)

Calcineurin (PP2B) is a phosphatase that inactivates CaMKII, thus returning the constitutive activity of CaMKII back to normal. In our AD model, the basal levels of PP2B were markedly upregulated (1.65 ± 0.216) in area CA1 (Figure 38). However, 4 weeks of treadmill exercise prevented the abnormal increase of PP2B in Aβ rats as the basal levels of Ex/Aβ rats (1.011 ± 0.12) were similar to those of control and exercise rats (control: 0.961 ± 0.075, exercise: 0.99 ± 0.068) (Figure 39).

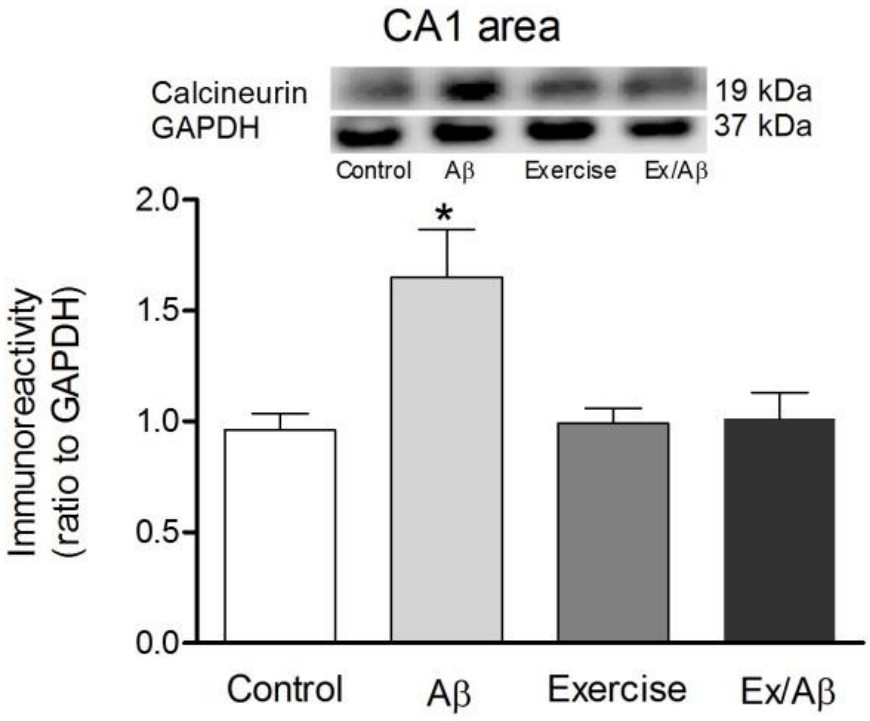

Figure 39. Basal levels of calcineurin (PP2B) in CA1 area. Exogenous administration of $A\beta_{1-42}$ peptides increases the basal levels of PP2B while moderate treadmill exercise prevents the aberrant upregulation of PP2B induced by AD pathology as the basal levels of PP2B of Ex/Aβ rats are similar to those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

Western blot analysis revealed that in DG area, there was an increase in the basal level of calcineurin (1.314 ± 0.399) and this increase was significant compared to other groups (p= 0.01-0.05). However, the basal level of calcineurin in DG area of Ex/Aβ rats (0.6516 ± 0.142) was similar to those of control (0.6704 ± 0.146) and exercise (0.7473 ± 0.1555) rats (Figure 40).

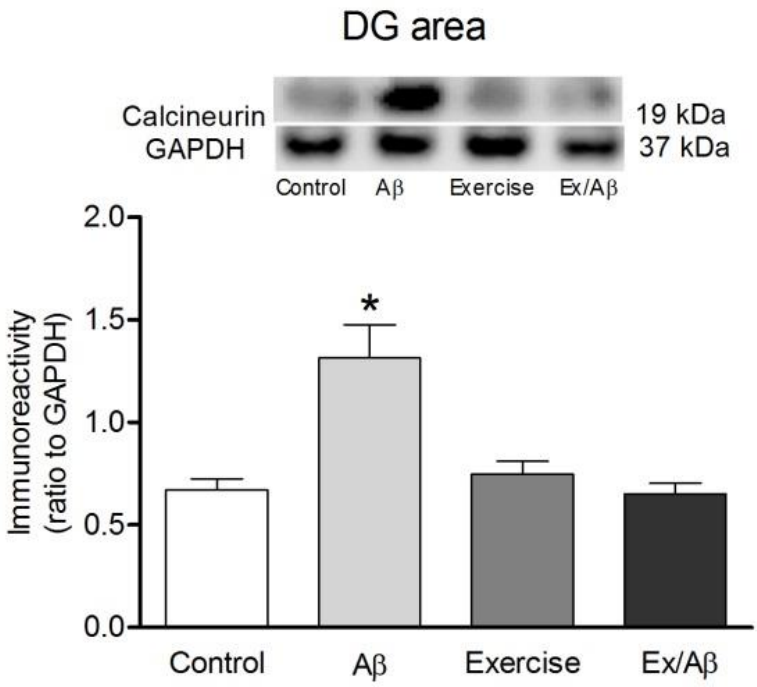

Figure 40. Basal levels of calcineurin (PP2B) in DG area. The basal level of PP2B is increased in Aβ rats while the basal levels of PP2B of Ex/Aβ rats are similar to those of control and exercise rats. Thus, the abnormal upregulation of PP2B induced by AD pathology is prevented by 4 weeks of moderate treadmill exercise. (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2.3. Basal levels of phosphorylated and total CREB

cAMP response element binding protein (CREB) is an important regulator of long-term memory and long-term synaptic plasticity (Kandel, 2012, Ran et al., 2012). Previously, we reported a reduction in basal levels of phosphorylated CREB associated

with AD pathology (Alkadhi et al., 2010). In this section, we investigated the ability of regular exercise to prevent the detrimental effects of AD on the basal levels of both phosphorylated (p-) and total (t-) CREB in CA1 and DG areas.

The basal level of p-CREB in CA1 area was markedly reduced in Aβ rats (0.633 ± 0.075, p = 0.05) and this reduction was antagonized by exercise as Ex/Aβ rats had a similar basal level of p-CREB compared to those of control and exercise rats (control: 1.102 ± 0.113, exercise: 1.34 ± 0.093, Ex/Aβ: 1.151 ± 0.176) (Figure 41A). The decrease in p-CREB level of Aβ rats is possibly responsible for the long-term memory deficit and L-LTP impairment in CA1 area of these rats. The basal levels of t-CREB in CA1 area among all groups were not different (control: 1.896 ± 0.094, Aβ: 1.962 ± 0.187, exercise: 2.14 ± 0.328, Ex/Aβ: 2.058 ± 0.107) (Figure 41B). As a result, the p-CREB : t-CREB ratio of Aβ rats in CA1 area was statistically lower compared to all other groups (control: 0.624 ± 0.094, Aβ: 0.3245 ± 0.0223, exercise: 0.7533 ± 0.1285, Ex/Aβ: 0.6447 ± 0.2212) (p= 0.01 - 0.05) (Figure 41C).

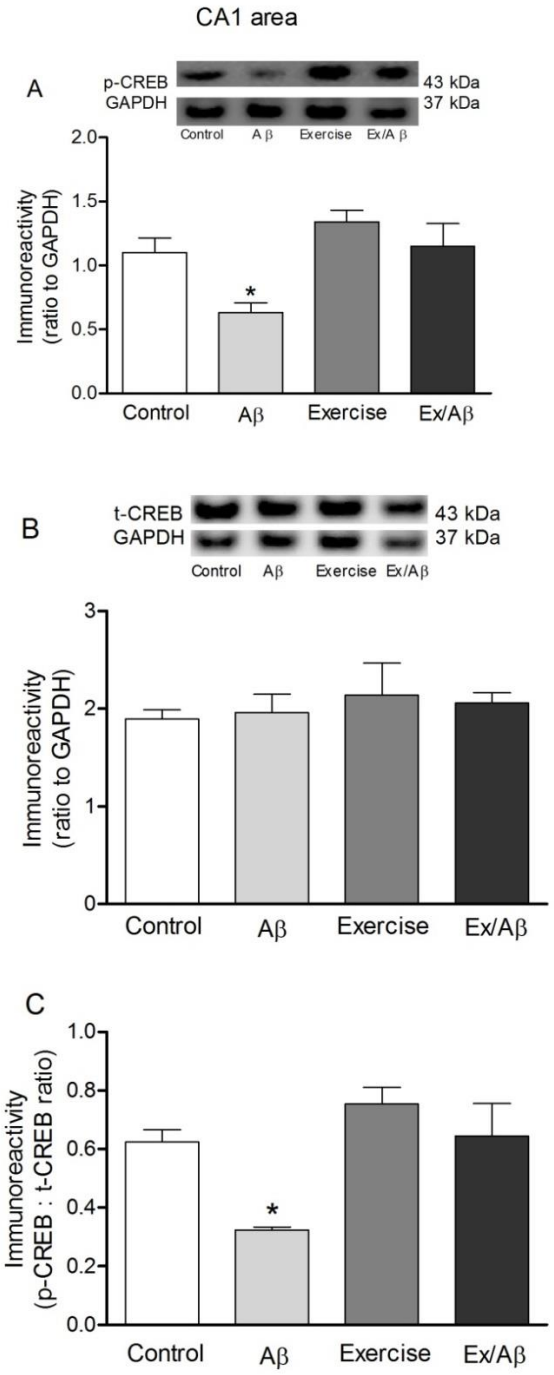

Figure 41. Basal levels of p-CREB (A), t-CREB (B), and p-CREB : t-CREB ratio (C) in CA1 area. The basal levels of p-CREB in Aβ rats are significantly reduced compared to other groups while the levels of t-CREB are similar across all groups. Thus, the p-CREB : t-CREB ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats, which indicates an impaired phosphorylation process. Regular treadmill exercise prevents amyloid infusion-induced reduction in basal levels of p-CREB and p-CREB : t-CREB ratio (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

Similar observation was found in the DG area. Moderate treadmill exercise prevented the decrease in basal level of p-CREB in DG area as this level in Ex/Aβ rats was 1.542 ± 0.3476, which was similar to those of control (1.332 ± 0.3161) and exercise (1.542 ± 0.1901) rats but significantly different from that of Aβ rats (0.899 ± 0.2753) (p= 0.001 – 0.05) (Figure 42A). The basal levels of t-CREB in DG area were unaltered among all groups (control: 1.976 ± 0.5127, Aβ: 1.856 ± 0.531, exercise: 1.811 ± 0.4626, Ex/Aβ: 1.949 ± 0.423) (Figure 42B). Therefore, the p-CREB : t-CREB ratio in DG area of Aβ rats was significantly lower than those of control, exercise, and Ex/Aβ rats (control: 0.7949 ± 0.2318, Aβ: 0.4454 ± 0.1443, exercise: 0.912 ± 0.1323, Ex/Aβ: 0.9257 ± 0.2681) (p= 0.01 – 0.05) (Figure 42C).

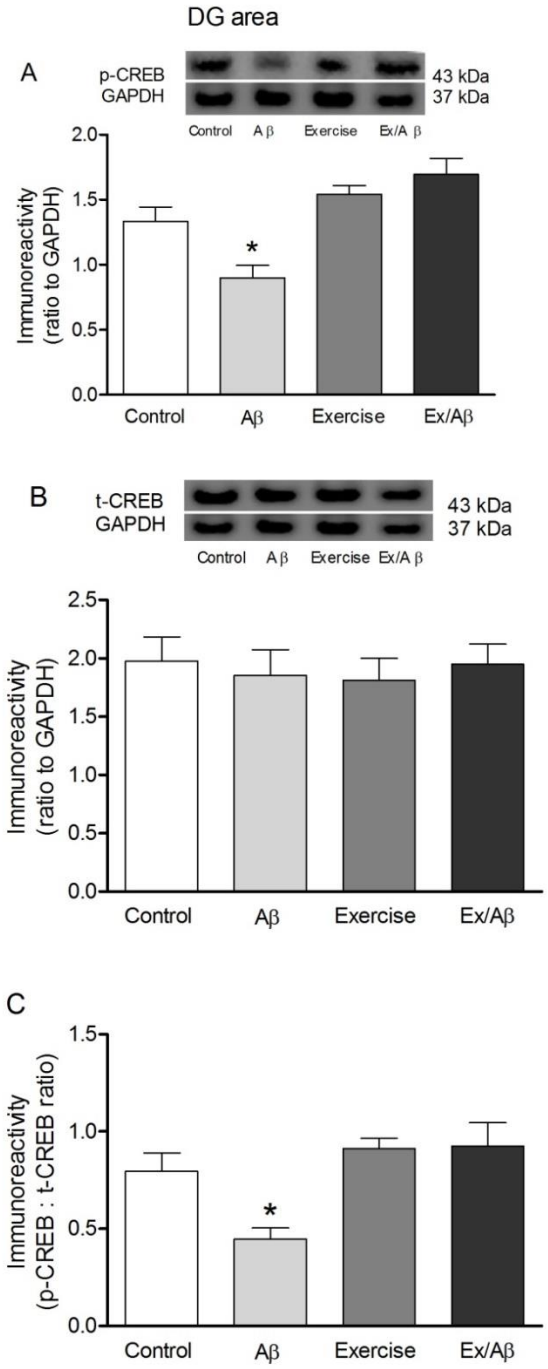

Figure 42. Basal levels of p-CREB (A), t-CREB (B), and p-CREB : t-CREB ratio (C) in DG area. The basal levels of p-CREB in Aβ rats are significantly reduced compared to other groups while the levels of t-CREB are similar across all groups. Thus, the p-CREB : t-CREB ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats. Regular treadmill exercise prevent impaired phosphorylation of p-CREB and reduction in p-CREB : t-CREB ratio caused by amyloid infusion. (*) indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2.4. Basal levels of CaMKIV

Ca^{2+}/calmodulin-dependent protein kinase IV is known to be involved in the CREB-related transcription process. In fact, CaMKIV activates CREB via phosphorylation at Ser133 and has been implicated in long-term memory consolidation and L-LTP expression (Kang et al., 2001, Kasahara et al., 2001, Smolen et al., 2006). Our findings indicated that the basal level of CaMKIV in CA1 area of Aβ rats was markedly decreased (p= 0.001) compared to all other groups (control: 0.9216 ± 0.2001, Aβ: 0.4485 ± 0.0815, exercise: 0.9746 ± 0.1501, Ex/Aβ: 1.068 ± 0.0712) (Figure 43).

Similarly, in DG area, the basal level of CaMKIV in Ex/Aβ rats was 0.6146 ± 0.1366, which was not different from those of control (0.7075 ± 0.050) and exercise (0.6914 ± 0.1479) rats but significantly higher than that of Aβ rats (0.2728 ± 0.0303) (p= 0.01 – 0.05) (Figure 43). Altogether, these results indicate the neuroprotective effect of moderate treadmill exercise against deleterious alterations that AD pathology has on total CaMKIV protein pool.

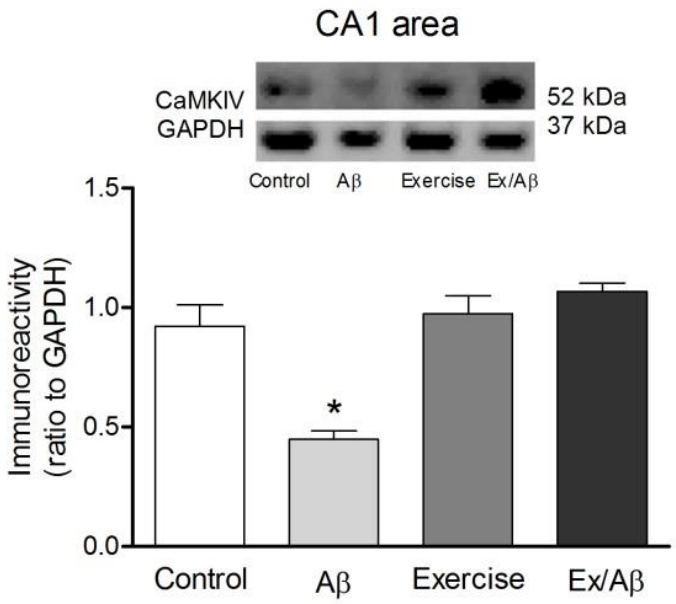

Figure 43. Western blot analysis of the basal levels of CaMKIV in the CA1 area. Continuous amyloid infusion decreases the basal level of CaMKIV and this reduction is prevented by treadmill exercise. The basal level of CaMKIV in Ex/Aβ rats is normal compared to those of control and exercise rats while this level is significantly lower in Aβ rats. (*) indicates significant difference from control, exercise, and Ex/Aβ groups, ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

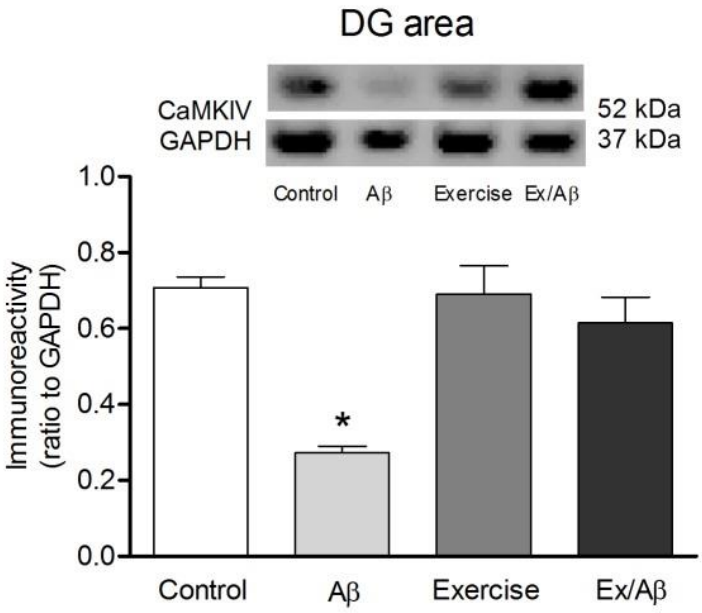

Figure 44. Basal levels of CaMKIV in DG area. In our rat model of Alzheimer's disease, the basal level of CaMKIV is markedly reduced and this deleterious reduction is prevented by 4 weeks of treadmill exercise. The basal level of CaMKIV in Ex/Aβ rats is similar to those of control and exercise rats. (*) indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2.5. Basal levels of phosphorylated and total ERK1/2

Extracellular signal-regulated kinases (ERK) signaling cascade has been shown to associate with cognitive brain function, particularly learning and memory process (Peng et al., 2010). In order to investigate the role of ERK1/2 signaling pathway in AD pathology and exercise, we measured the basal levels of both phosphorylated (p-) and total (t-) ERK1/2 in the hippocampus. Surprisingly, our data revealed that in CA1, the basal levels of p-ERK1/2 remained unchanged across all groups (control: 1.0 ± 0.2659, Aβ: 0.9245 ± 0.3049, exercise: 1.145 ± 0.3389, Ex/Aβ: 0.9538 ± 0.3393) (Figure 45A). Also, neither amyloid infusion and/or exercise training altered the basal level of t-ERK1/2 in CA1 area as this level was similar among all groups (control: 1.0 ± 0.204, Aβ: 0.8983 ± 0.1512, exercise: 1.086 ± 0.3048, Ex/Aβ: 0.8713 ± 0.2212) (Figure 45B). As a result, the p-ERK1/2 : t-ERK1/2 ratios of control (1.025 ± 0.3046), Aβ (1.086 ± 0.5007), exercise (0.9514 ± 0.3404), Ex/Aβ (1.098 ± 0.29) were not different (Figure 45C).

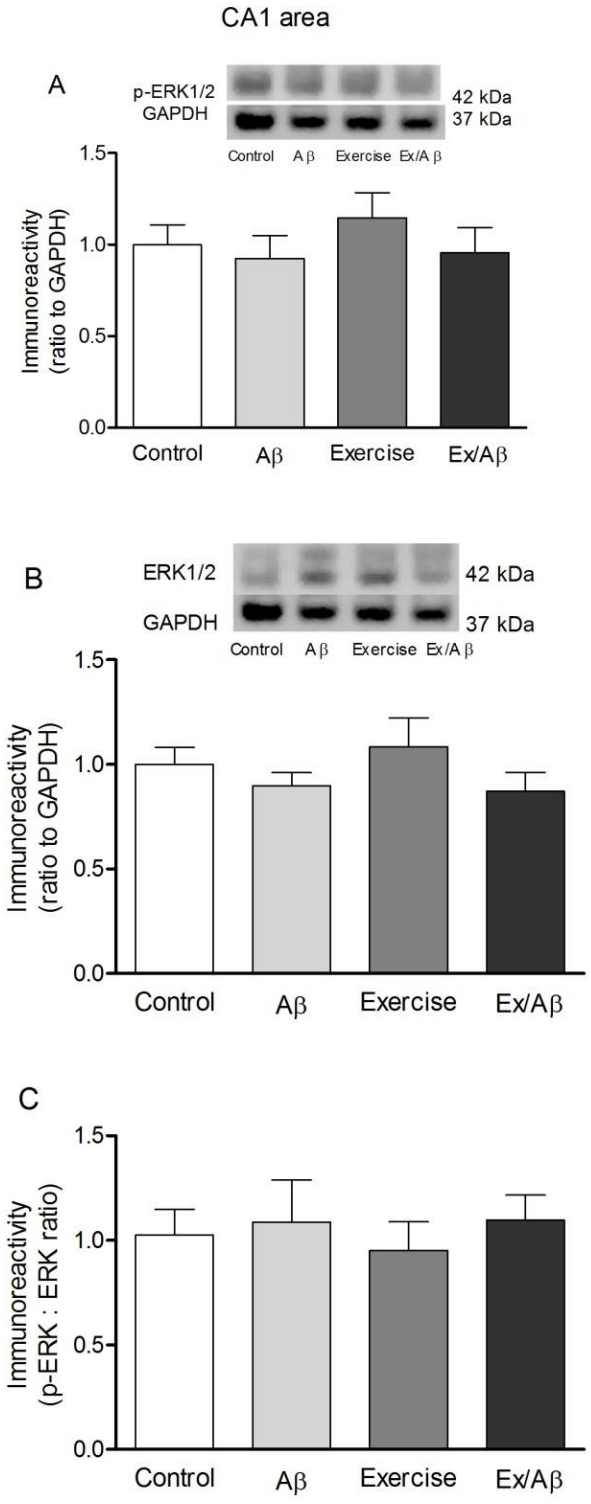

Figure 45. Basal levels of p-ERK1/2 (A), t-ERK1/2 (B), and p-ERK1/2 : t-ERK1/2 ratio (C) in CA1 area. There are no differences in the basal levels of p-ERK1/2, t-ERK1/2, and p-ERK1/2 : t-ERK1/2 ratio across all groups. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

Western blot analysis of DG total homogenates indicated the same trend seen in the CA1 area. The basal levels of p-ERK1/2 in the DG sub-region for control, Aβ, exercise, and Ex/Aβ were 0.9675 ± 0.2414, 0.8819 ± 0.2531, 0.9661 ± 0.1503, 0.9651 ± 0.1201 respectively (Figure 46A). The total pool of ERK1/2 protein in DG area was also unaffected by amyloid infusion or treadmill exercise (control: 1.0 ± 0.23, Aβ: 0.9632 ± 0.2611, exercise: 0.9503 ± 0.2178, Ex/Aβ: 0.8446 ± 0.1157) (Figure 46B). Thus, the p-ERK1/2 : t-ERK1/2 ratios of all groups were closely similar (control: 1.027 ± 0.1432, Aβ: 0.9004 ± 0.3077, exercise: 0.9595 ± 0.1442, Ex/Aβ: 1.037 ± 0.2296) (Figure 46C).

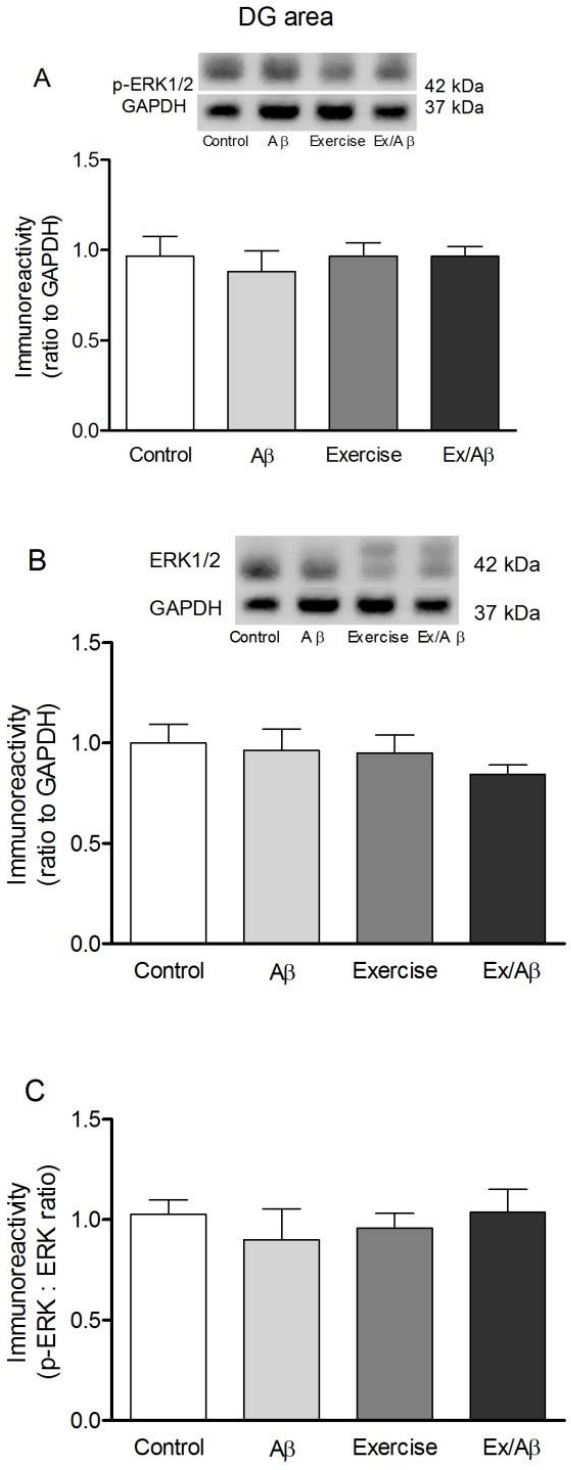

Figure 46. Basal levels of p-ERK1/2 (A), t-ERK1/2 (B), and p-ERK1/2 : t-ERK1/2 ratio (C) in CA1 area. There are no differences in the basal levels of p-ERK1/2, t-ERK1/2, and p-ERK1/2 : t-ERK1/2 ratio across all groups. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots.

4.3.2.6. Basal levels of BDNF

Brain-derived neurotrophic factor (BDNF) can exert a pleiotrophic effect in the central nervous system as BDNF can affect neuronal growth, synaptic transmission and plasticity as well as pathogenesis of psychiatric disorders (Hong et al., 2011). While the basal level of BDNF in Aβ rats was similar to that of controls, those of both exercise and Ex/Aβ rats were significantly increased compared to that of control rats (control: 1 ± 0.048, Aβ: 0.833 ± 0.071, exercise: 1.568 ± 0.136, Ex/Aβ: 1.565 ± 0.141, p = 0.01) (Figure 47). Perhaps, exercise-induced BDNF increase might be beneficial to prevent behavioral disturbances caused by AD pathology.

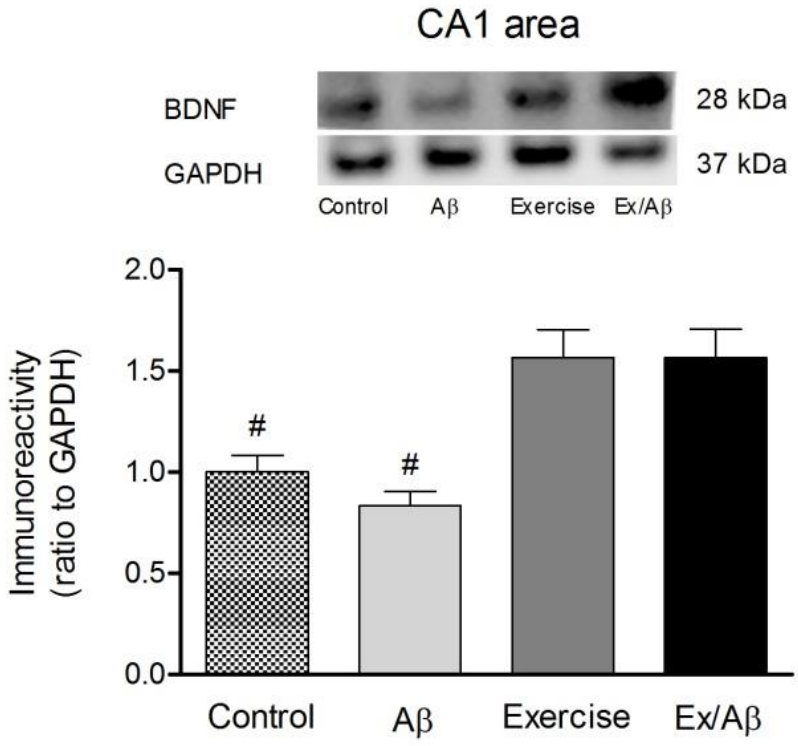

Figure 47. Basal levels of brain-derived neurotrophic factor (BDNF) in CA1 area. Exogenous administration of $A\beta_{1-42}$ peptides does not significantly affect BDNF levels. Moderate treadmill exercise significantly upregulates the basal levels of BDNF in exercised animals including Ex/Aβ rats. Values are mean ± S.E.M., n = 4-6 rats/group. # indicates significant difference from exercise and Ex/Aβ rats ($p < 0.05$). Insets are representative blots.

The dentate gyrus (DG) is one of the brain regions in which neurogenesis occurs. This critical area of the brain houses numerous neurotrophic factors including BDNF. Our data revealed that in DG area, regular exercise significantly increased the basal levels of BDNF in exercised rats (exercise: 1.758 ± 0.5121, Ex/Aβ: 1.806 ± 0.6664). However, the basal level of BDNF was similar to that of control sedentary controls (control: 1.0 ± 0.1601, Aβ: 0.9145 ± 0.1682) (Figure 48).

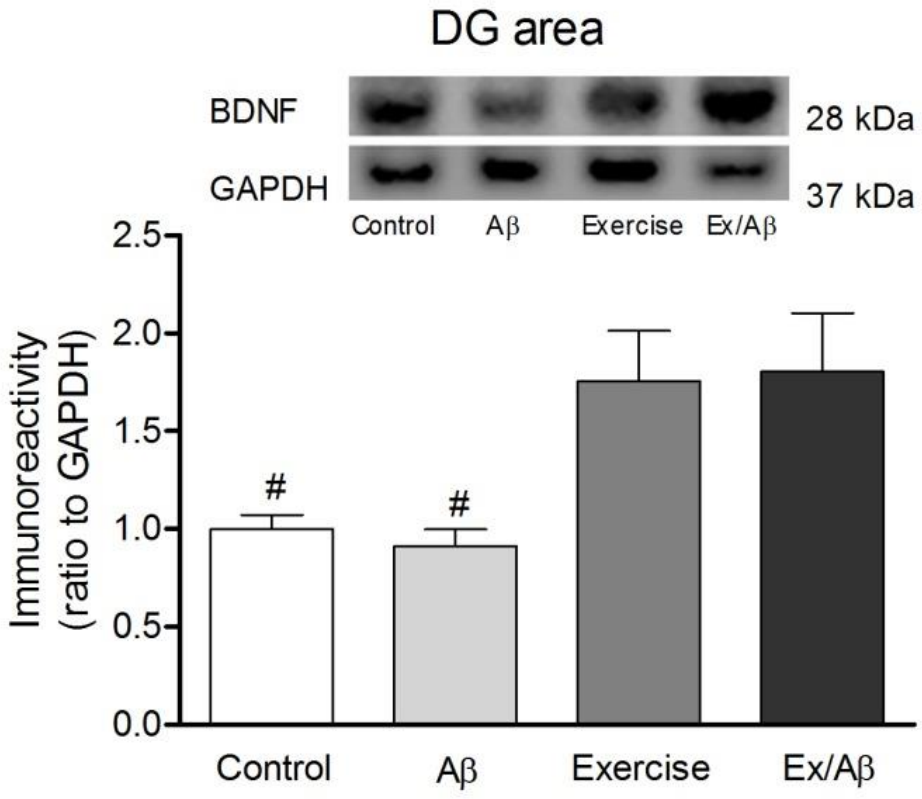

Figure 48. Basal levels of brain-derived neurotrophic factor (BDNF) in DG area. Exogenous administration of $A\beta_{1-42}$ peptides does not affect BDNF levels. Moderate treadmill exercise significantly upregulates the basal levels of BDNF in exercised animals including Ex/Aβ rats. Values are mean ± S.E.M., n = 4-6 rats/group. (#) indicates significant difference from exercise and Ex/Aβ rats ($p < 0.05$). Insets are representative blots.

4.3.3. Levels of signaling molecules during E-LTP in CA1 area

4.3.3.1. Levels of phosphorylated and total CaMKII after E-LTP expression in CA1 area

After activity i.e. E-LTP, the levels of p-CaMKII increased in control animals. We reported that in CA1 area, the levels of phosphorylated (p)-CaMKII in unstimulated control (1.0 ± 0.152) were significantly different compared to those of stimulated (S-) Control, S-Exercise, and S-Ex/Aβ groups (S-Control: 1.575 ± 0.152, S-Ex: 1.889 ± 0.153, S-Ex/Aβ: 1.825 ± 0.18, p= 0.01-0.05) but were not significantly different from those of S-Aβ rats (0.578 ± 0.122) (Figure 49A). Total (t)-CaMKII levels were similar across all stimulated groups in being markedly higher than those of unstimulated control (unstimulated control: 0.956 ± 0.028, S-Control: 1.404 ± 0.096, S-Aβ: 1.301 ± 0.138, S-Exercise: 1.648 ± 0.175, S-Ex/Aβ: 1.453 ± 0.137, p= 0.05 (Figure 49B). This result indicates that CaMKII phosphorylation is inhibited in Aβ rats, which is prevented by treadmill exercise. Furthermore, it is supported by the finding that the ratio of p-CaMKII: t-CaMKII was markedly lower in S-Aβ rats compared to all other stimulated groups and even the unstimulated control (p=0.01-0.05, Figure 49C). These data suggest that although the total pool of CaMKII is unaltered, phosphorylation of this protein is negatively affected and that this effect is prevented by 4 weeks of treadmill exercise.

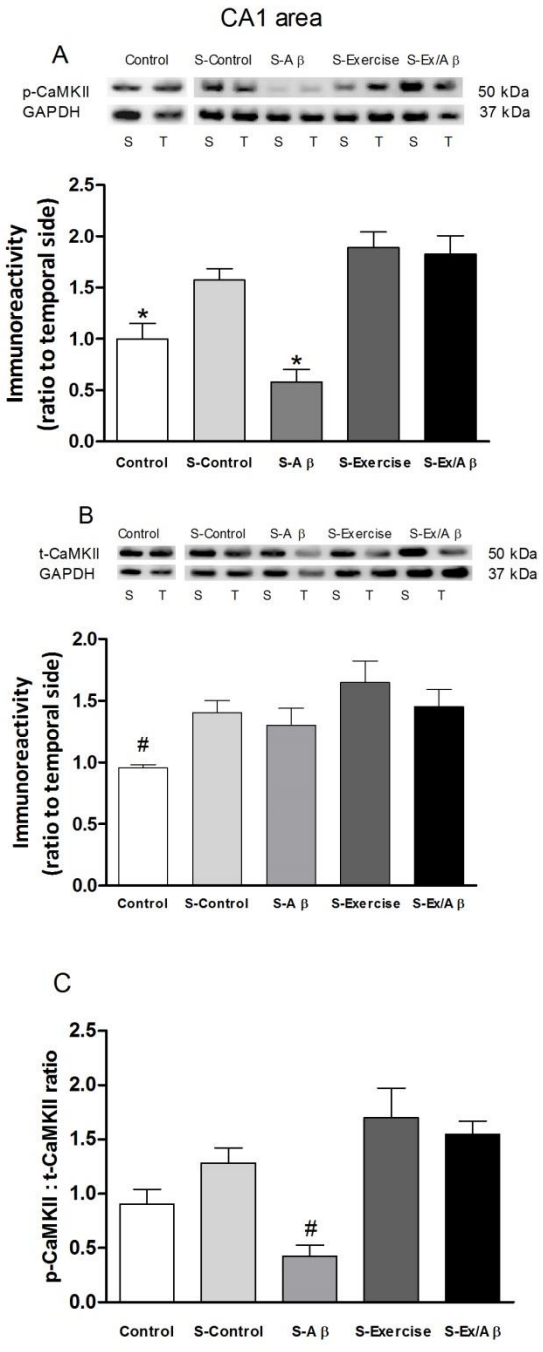

Figure 49. Levels of phosphorylated (p)-CaMKII (A), total (t)-CaMKII (B), and the p-CaMKII:t-CaMKII ratio (C) in area CA1 after E-LTP induction. Compared to the unstimulated control group, HFS increases the level of p-CaMKII in all groups except the Aβ rats and significantly increases the levels of t-CaMKII in all groups. Thus, after HFS, the p-CaMKII : t-CaMKII ratio is significantly lowered in Aβ rats compared to all other groups including unstimulated control rats. Regular exercise prevents AD-induced reduction in p-CaMKII level. (*) indicates significant difference from stimulated (S)-Control, S-Exercise, and S-Ex/Aβ, (#) indicates significant difference from all other groups, p= 0.01- 0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots. S: septal portion, T: temporal portion of CA1 area.

4.3.3.2. Levels of calcineurin after E-LTP expression in CA1 area

Calcineurin (PP2B) is a protein phosphatase important for dephosphorylation of CaMKII. One hour after HFS application, the level of calcineurin in S-Control (1.404 ± 0.107) and S-Aβ (1.51 ± 0.117) rats increased significantly compared to unstimulated control (0.913 ±0.098) (p= 0.05). In contrast, the levels of calcineurin after HFS in S-Ex (0.995 ± 0.08) and S-Ex/Aβ (0.974 ±0.113) rats were similar to those in unstimulated controls suggesting that exercise prevents upregulation of PP2B by HFS (Figure 50).

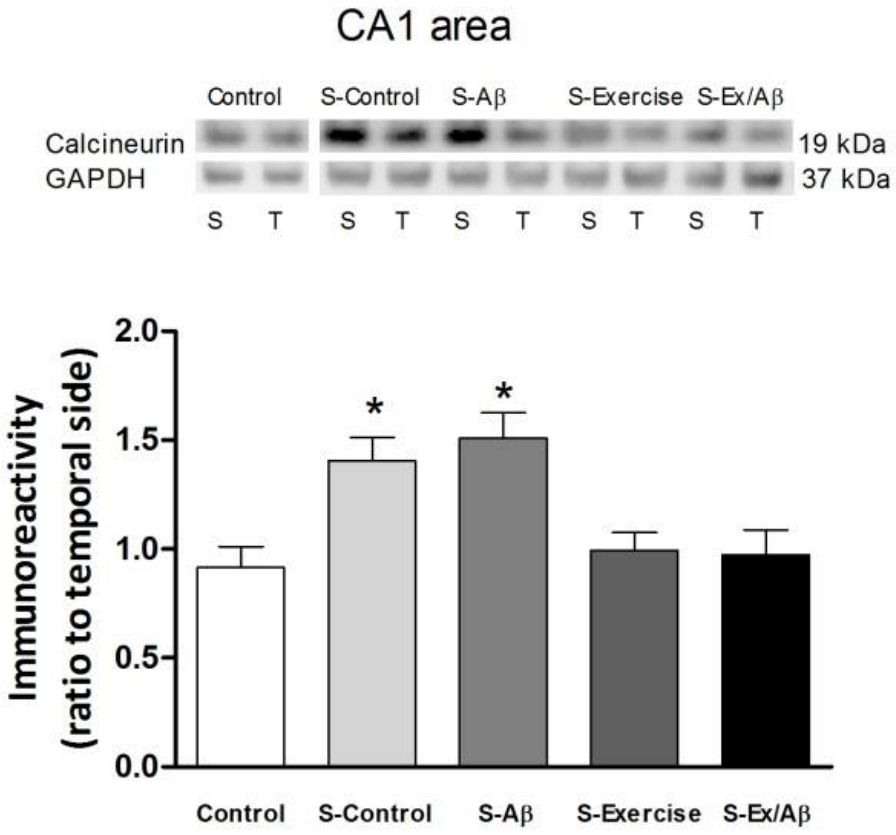

Figure 50. Levels of calcineurin in area CA1 after E-LTP induction. HFS application increases the expression of calcineurin in S-Control and S-Aβ rats which are significant higher than those of S-Exercise, S-Ex/Aβ, and unstimulated control rats. (*) indicates significant difference from unstimulated control, S-Exercise, and S-Ex/Aβ, p =0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion of CA1 area.

4.3.3.3. Levels of BDNF after E-LTP expression in CA1 area

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor critical for neurogenesis, cognition, and synaptic plasticity (Vivar et al., 2013). BDNF can exist in both monomeric and homodimeric forms in cells (Shen and Maruyama, 2012). The present protocol of HFS of the Schaffer collateral synapses did not appreciably increase the levels of BDNF in S-Control (monomer level: 1.084 ± 0.103, dimer level: 1.087 ± 0.098) and S-Aβ rats (monomer level: 1.093 ± 0.116, dimer level: 1.149 ± 0.134). However, treadmill exercise significantly increased both BDNF monomer (Figure 51A) and dimer protein (Figure 51B) levels in exercised animals including S-Ex (monomer level: 1.679 ± 0.179, dimer level: 1.854 ± 0.195) and S-Ex/Aβ rats (monomer level: 1.696 ± 0.193, dimer level: 2.012 ± 0.398) compared to unstimulated control rats (monomer level: 1.005 ± 0.049, dimer level: 1.149 ± 0.134) (p= 0.01 - 0.05). Thus, in our rat model of AD, treadmill exercise markedly up-regulated both forms of BDNF, even in the presence of Aβ (Figure 51 A,B).

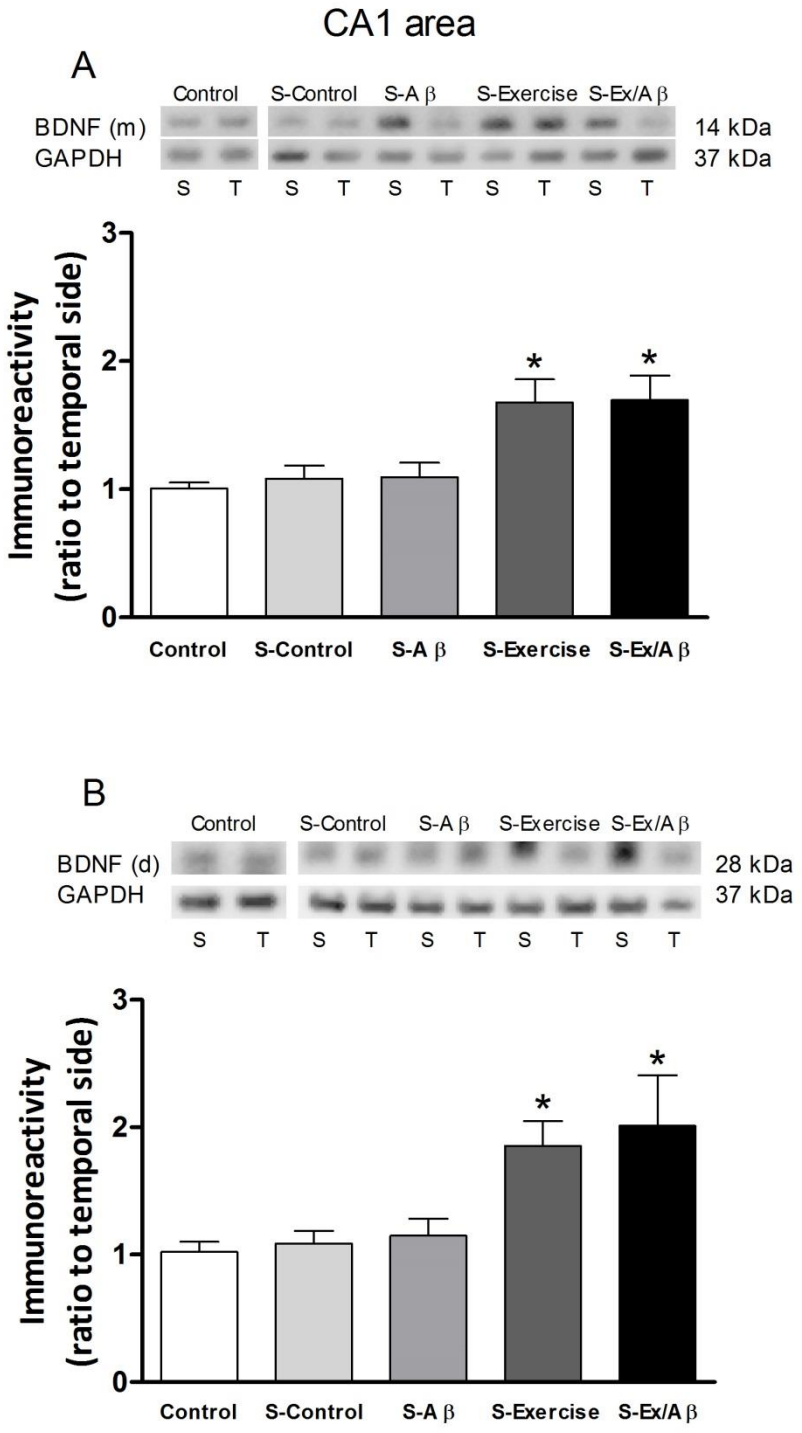

Figure 51. Monomeric (m) (A, 14 kDa) and dimeric (d) (B, 28KDa) BDNF in area CA1 after HFS induction. HFS does not increase the level of BDNF monomers or dimers in S-Control and S-Aβ rats but significantly elevates those of S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from unstimulated control, S-Control, and S-Aβ, p = 0.01 – 0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion of CA1 area.

4.3.4. Levels of signaling molecules during E-LTP in DG area

4.3.4.1. Levels of phosphorylated and total CaMKII after E-LTP expression in DG area

In the DG area, we found that single train of HFS robustly increased the level of p-CaMKII in S-Control (1.629 ± 0.115), S-Exercise (1.911 ± 0.238), and S-Ex/Aβ rats (2.023 ± 0.268) compared to unstimulated control (1.03 ± 0.151) (p= 0.01 – 0.05) (Figure 52A). However, the same E-LTP induction protocol failed to appreciably increase the p-CaMKII level in Aβ rats (0.603 ± 0.175). After HFS the levels of t-CaMKII in stimulated groups were equally elevated (S-Control: 1.779 ± 0.5043, S-Aβ: 1.731 ± 0.292, S-Exercise: 1.965 ± 0.4194, and S-Ex/Aβ: 1.869 ± 0.6925) but compared to that of the unstimulated control (0.8717 ± 0.2421) (p= 0.05 – 0.01) (Figure 52B). As a result, the p-CaMKII : t-CaMKII ratio in DG area of S-Aβ rats (0.3933 ± 0.2784) was significantly lower than those of all other groups (S-Control: 1.223 ± 0.1201, S-Exercise: 1.432 ± 0.3682, and S-Ex/Aβ: 1.256 ± 0.2239) including unstimulated control (1.041 ± 0.3477) (p= 0.01 - 0.05) (Figure 52C).

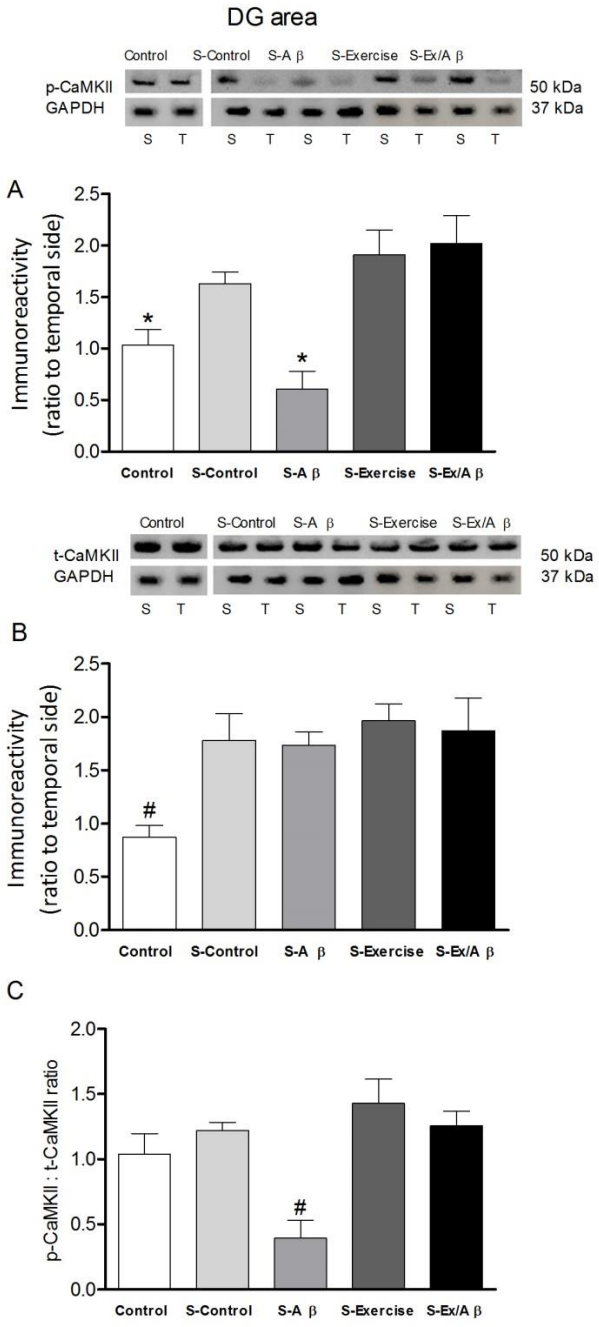

Figure 52. Levels of phosphorylated (p)-CaMKII (A), total (t)-CaMKII (B), and the p-CaMKII : t-CaMKII ratio (C) in the DG area after E-LTP induction. Compared to the unstimulated control group, HFS increases the level of p-CaMKII in all groups except the Aβ rats and significantly increased the levels of t-CaMKII in all groups. Thus, after HFS, the p-CaMKII : t-CaMKII ratio was significantly lowered in Aβ rats compared to all other groups including unstimulated control rats. Regular exercise prevented AD-induced reduction in p-CaMKII level. (*) indicates significant difference from stimulated (S)-Control, S-Exercise, and S-Ex/Aβ, (#) indicates significant difference from all other groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots. S: septal portion, T: temporal portion of DG area.

4.3.4.2. Levels of calcineurin after E-LTP expression in DG area

There was an increased levels of calcineurin (PP2B) after E-LTP induction via HFS in the perforant synapses in S-Control (1.601 ± 0.3312) and S-Aβ (1.539 ± 0.3317) rats compared to unstimulated control (1.017 ± 0.1626) (p= 0.05) (Figure 53). The levels of PP2B during E-LTP expression in the DG area of exercised rats including the Ex/Aβ group were similar to that of unstimulated control (S-Exercise: 1.041 ± 0.4078, S-Ex/Aβ: 1.083 ± 0.2347).

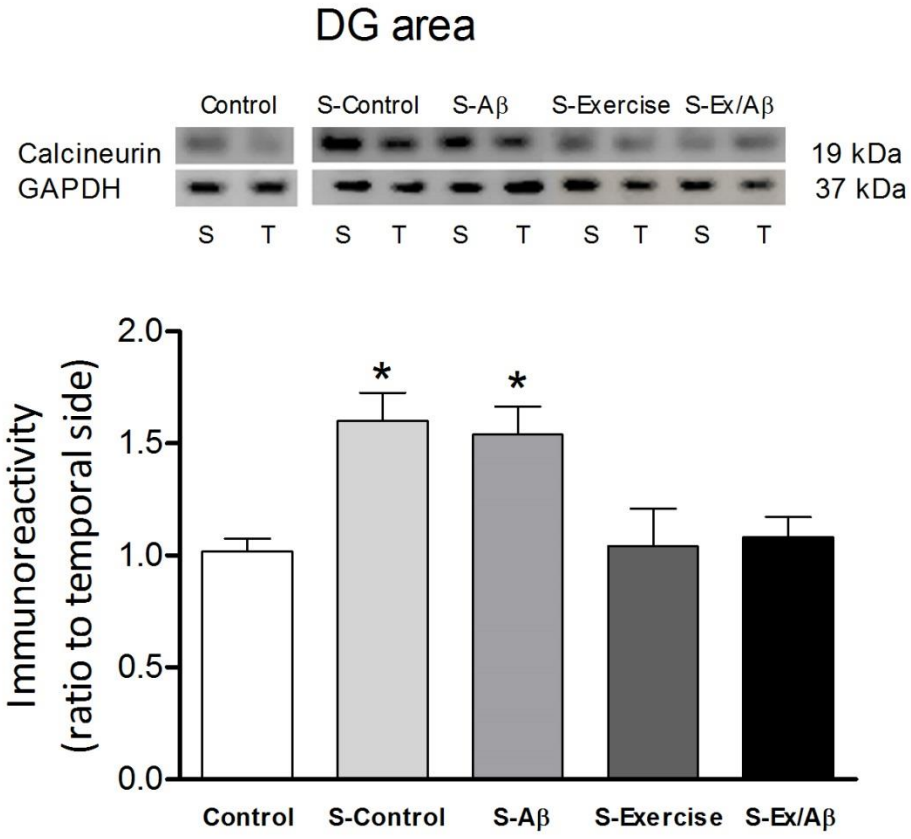

Figure 53. Levels of calcineurin in DG area after E-LTP induction. HFS application increases the expression of calcineurin in S-Control and S-Aβ rats which are significantly higher than those of S-Exercise, S-Ex/Aβ, and unstimulated control rats. (*) indicates significant difference from unstimulated control, S-Exercise, and S-Ex/Aβ, p =0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion of the hippocampal DG area.

4.3.4.3. Levels of BDNF after E-LTP expression in DG area

Neurogenesis is known to occur in the DG sub-region of the hippocampus, thus making this area a storage pool of neurotrophins including BDNF. In this section, we examined the effect of AD pathology and/or moderate treadmill exercise on the levels of BDNF in the DG area after E-LTP expression. We found that exercise and/or amyloid infusion exerted similar effects on levels of BDNF monomer and dimer in CA1 area. We suspect that similar trend will be observed in DG area, thus we measured only BDNF dimers levels in the DG area after E-LTP induction. Our data showed that HFS significantly up-regulated the BDNF levels in S-Exercise (2.315 ± 0.6279) and S-Ex/Aβ (2.067 ± 0.5055) groups compared to unstimulated control (1.0 ± 0.1959), S-Control (1.196 ± 0.4989), and S-Aβ (1.155 ± 0.5027) groups ($p = 0.05$) (Figure 54). Thus, it seems that BDNF might be a potential factor in the mechanism behind the beneficial effect of exercise on cognition.

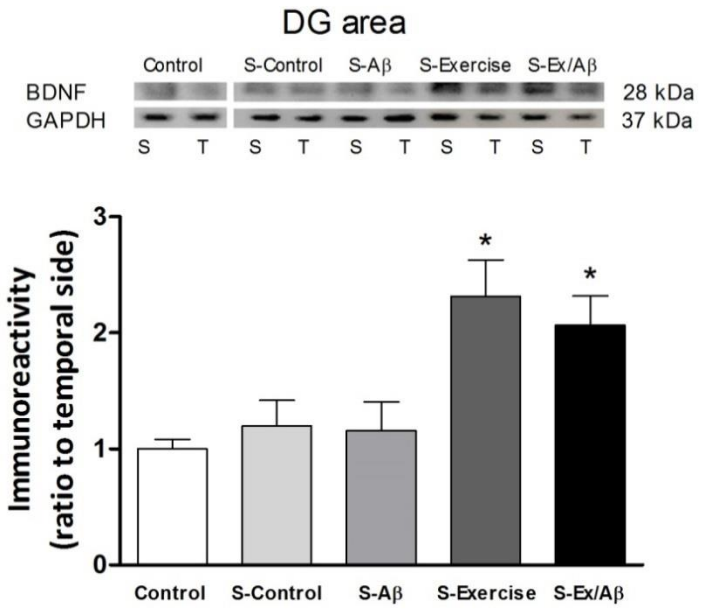

Figure 54. Levels of BDNF after HFS induction in DG area. HFS does not increase the level of BDNF in S-Control and S-Aβ rats but significantly elevates those of S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from unstimulated control, S-Control, and S-Aβ, p = 0.01 – 0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion.

4.3.5. Levels of signaling molecules during L-LTP in CA1 area

4.3.5.1. Levels of phosphorylated and total CREB after L-LTP expression in CA1 area

Long-lasting changes at the synapses and L-LTP expression depend heavily on the activation status of CREB via phosphorylation (Bengtson and Bading, 2012). In this section, we investigated the influence of amyloid infusion and/or exercise training on the activity-dependent Schaffer collateral synapses. Compared to unstimulated control (0.9995 ± 0.0554), in CA1 area multiple train of HFS (MHFS) failed to increase the level of p-CREB in S-Aβ rats (0.8082 ± 0.2027) as seen in other groups (S-Control: 1.536 ± 0.3153, S-Exercise: 1.554 ± 0.3199, S-Ex/Aβ: 1.697 ± 0.2415) (p= 0.001 - 0.01) (Figure 55A). In contrast, 5 hrs after L-LTP induction, the levels of t-CREB in all stimulated groups were similar (S-Control: 1.711 ± 0.3665, S-Aβ: 1.991 ± 0.3188, S-Exercise: 2.051 ± 0.5934,

S-Ex/Aβ: 1.748 ± 0.4387) but markedly higher than that of unstimulated control (0.9973 ± 0.0604) (p= 0.01 – 0.05) (Figure 55B). Thus, the p-CREB : t-CREB ratio of S-Aβ rats (0.3908 ± 0.1744) was much lower than those of stimulated (S-Control: 1.043 ± 0.3672, S-Exercise: 0.9333 ± 0.3673, S-Ex/Aβ: 1.005 ± 0.2468) and unstimulated control (1.004 ± 0.1004) groups (p= 0.01 - 0.05) (Figure 55C). Together, these findings indicated that AD pathology impaired stimulation-induced CREB phosphorylation process and this impairment was prevented by prior exercise.

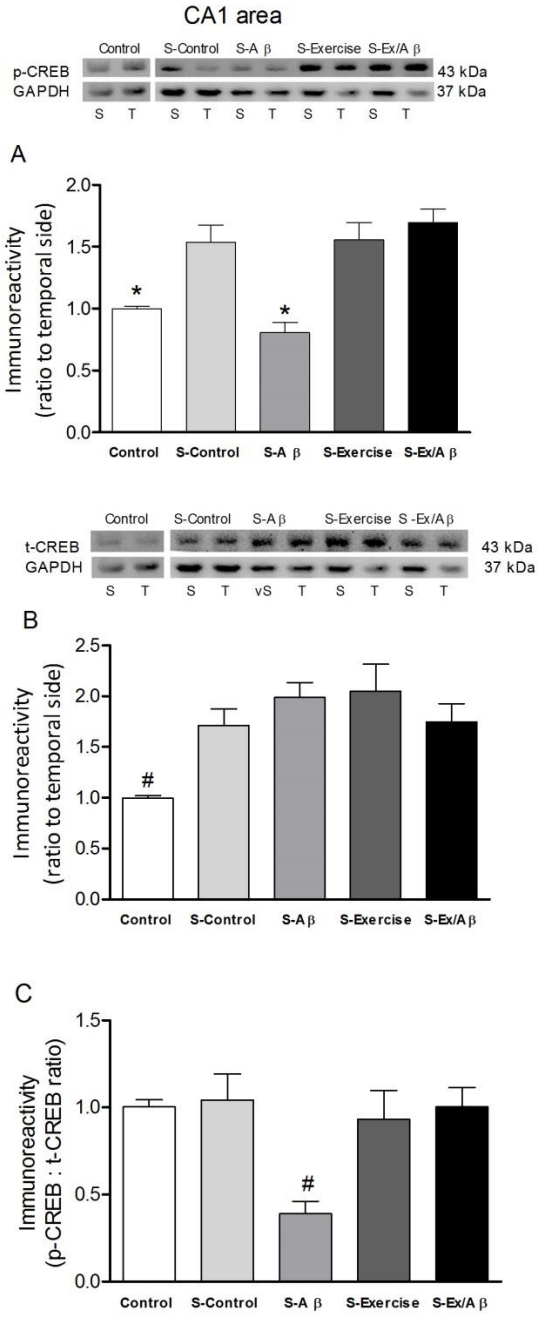

Figure 55. Levels of phosphorylated (p)-CREB (A), total (t)-CREB (B), and the p-CREB : t-CREB ratio (C) in the CA1 area after L-LTP induction. Compared to the unstimulated control group, MHFS increases the level of p-CREB in all groups except the Aβ rats and significantly increases the levels of t-CREB in all groups compared to unstimulated controls. Thus, after MHFS, the p-CREB : t-CREB ratio is significantly lower in Aβ rats compared to all other groups including unstimulated control rats. Regular exercise prevents AD-induced reduction in p-CREB level. (*) indicates significant difference from stimulated (S)-Control, S-Exercise, and S-Ex/Aβ, (#) indicates significant difference from all other groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots. S: septal portion, T: temporal portion of CA1 area.

4.3.5.2. Levels of CaMKIV after L-LTP expression in CA1 area

Five hours after L-LTP induction via MHFS of the Schaffer/collaterals pathway, the levels of CaMKIV were significantly increased in S-Control (1.835 ± 0.5041), S-Exercise (2.099 ± 0.4852), and S-Ex/Aβ (1.842 ± 0.2535) rats compared to unstimulated control (1.191 ± 0.1246) (p= 0.01 - 0.05). However, the MHFS-induced increase in CaMKIV level in the CA1 area was absent in S-Aβ rats (0.7295 ± 0.3429) (Figure 56).

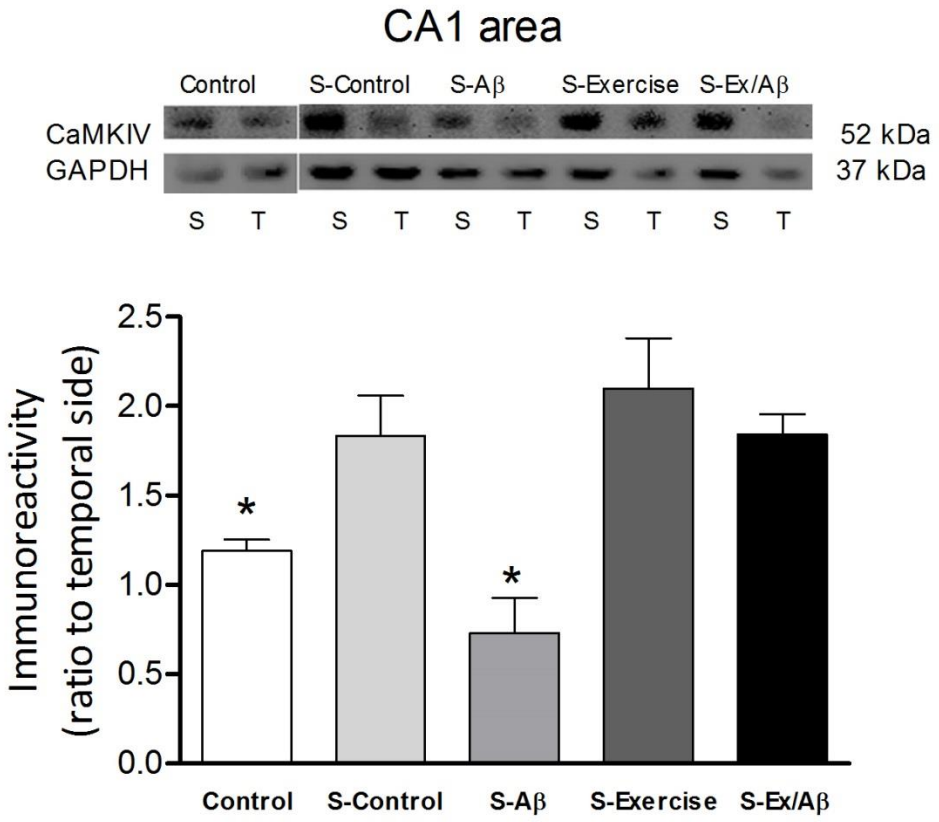

Figure 56. Levels of CaMKIV in area CA1 after L-LTP induction via MHFS. MHFS fails to increase the level of CaMKIV S-Aβ rats but significantly elevates those of S-Control, S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from S-Control, and S-Exercise and S-Ex/Aβ groups, p = 0.01 – 0.05. Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion.

4.3.5.3. Levels of BDNF after L-LTP expression in CA1 area

In this section, we investigated the effect of exercise in AD pathology by measuring the level of BDNF in CA1 area after L-LTP induction. Multiple trains of HFS significantly elevated BDNF levels in all animals (S-Control: 1.808 ± 0.8429, S-Exercise: 1.755 ± 0.5874, S-Ex/Aβ: 1.482 ± 0.4118) except S-Aβ rats (0.7847 ± 0.2873) compared to unstimulated control (0.8265 ± 0.2566) ($p= 0.05$) (Figure 57). Perhaps by increasing the availability of BDNF, which involves in long-lasting changes at the synapses, regular treadmill exercise is able to prevent deleterious alterations of BDNF downstream regulators such as CREB and CaMKIV as reported in our study.

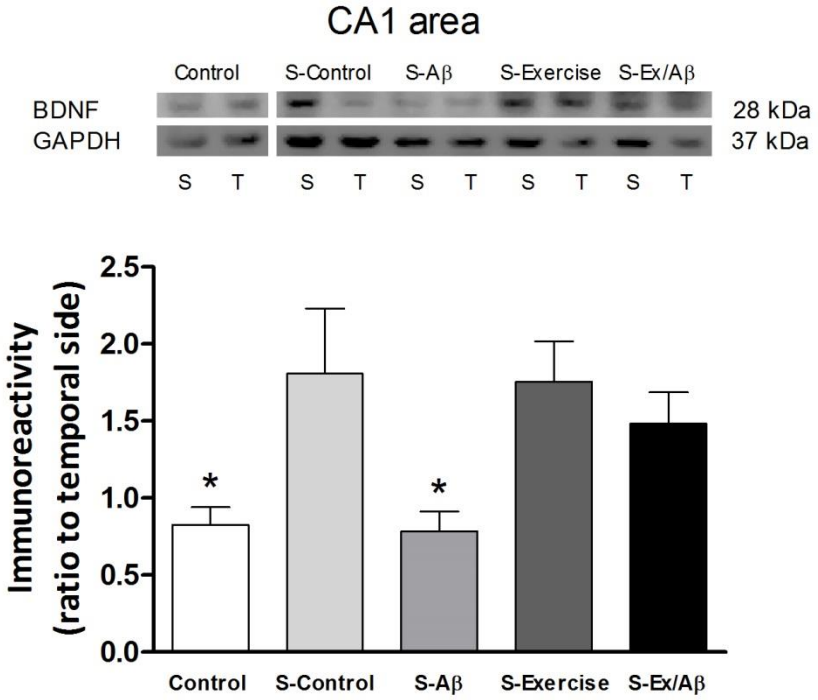

Figure 57. Levels of BDNF in the Schaffer collateral pathway after MHFS application. MHFS fails to increase the level of BDNF in S-Aβ rats but significantly elevates those of S-Control, S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from S-Control, and S-Exercise and S-Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion of the CA1 area.

4.3.6. Levels of signaling molecules during L-LTP in DG area

4.3.6.1. Levels of phosphorylated and total CREB after L-LTP expression in DG area

As CREB is a transcription factor regulating neurotrophic factors synthesis, CREB is known to play a role in L-LTP expression in the DG area where neurogenesis takes place during and after development. Thus, in this section, we evaluated the changes in CREB levels in AD pathology and/or exercise training 5 hours after induction of L-LTP in the DG area. Compared to unstimulated controls (0.8951 ± 0.1984), MHFS induced a marked increase in levels of p-CREB in S-Control (1.451 ± 0.4355), S-Exercise (1.466 ± 0.167), S-Ex/Aβ (1.541 ± 0.3384) but failed to produce the same effect in S-Aβ rats

(0.7651 ± 0.2018) (p= 0.05) (Figure 58A). Our L-LTP protocol resulted in equally increased levels of t-CREB among all stimulated groups (S-Control: 1.798 ± 0.3589, S-Aβ: 1.855 ± 0.1114, S-Exercise: 1.868 ± 0.4975, S-Ex/Aβ: 1.81 ± 0.4599) compared to the unstimulated control group (0.887 ± 0.218) (p= 0.01 - 0.05) (Figure 58B). Therefore, the p-CREB : t-CREB ratio of S-Aβ rats (0.3692 ± 0.1536) was significantly lower than all other groups (unstimulated control: 0.9815 ± 0.3242, S-Control: 0.9261 ± 0.276, S-Exercise: 0.778 ± 0.1751, S-Ex/Aβ: 0.9344 ± 0.2832) (p= 0.05) (Figure 58C).

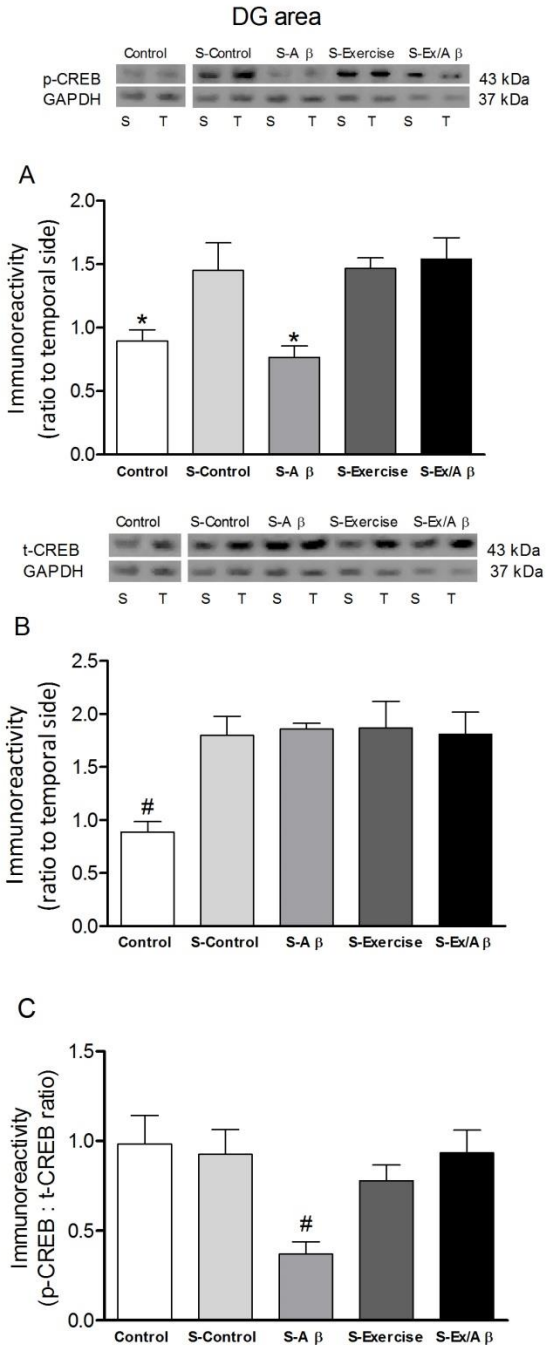

Figure 58. Levels of phosphorylated (p)-CREB (A), total (t)-CREB (B), and the p-CREB : t-CREB ratio (C) in the DG area after L-LTP induction. Compared to the unstimulated control group, MHFS increases the level of p-CREB in all groups except the Aβ rats and significantly increases the levels of t-CREB in all groups. Thus, after MHFS, the p-CREB : t-CREB ratio is significantly lower in Aβ rats compared to all other groups including unstimulated control rats. Regular exercise prevents AD-induced reduction in p-CREB level. (*) indicates significant difference from stimulated (S)-Control, S-Exercise, and S-Ex/Aβ, (#) indicates significant difference from all other groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative blots. S: septal portion, T: temporal portion of the DG area.

4.3.6.2. Levels of CaMKIV after L-LTP expression in DG area

Multiple trains of repetitive HFS induces L-LTP in the perforant synapses creating long-lasting changes in the biochemical pathway, one of such is CaMKIV, a kinase that phosphorylates CREB. Our data indicated that 5 hrs after L-LTP expression in the DG area, the levels of CaMKIV were markedly elevated in S-Control (2.328 ± 0.324), S-Exercise (2.521 ± 0.2638) and S-Ex/Aβ (2.565 ± 0.5888) rats compared to unstimulated control rats (1.577 ± 0.1521) ($p= 0.05$). In contrast, MHFS failed to appreciably increase the level of CaMKIV in Aβ rats (1.52 ± 0.5949) which could possibly explain the lower p-CREB level in S-Aβ rats after L-LTP expression compared to all other stimulated groups (Figure 59).

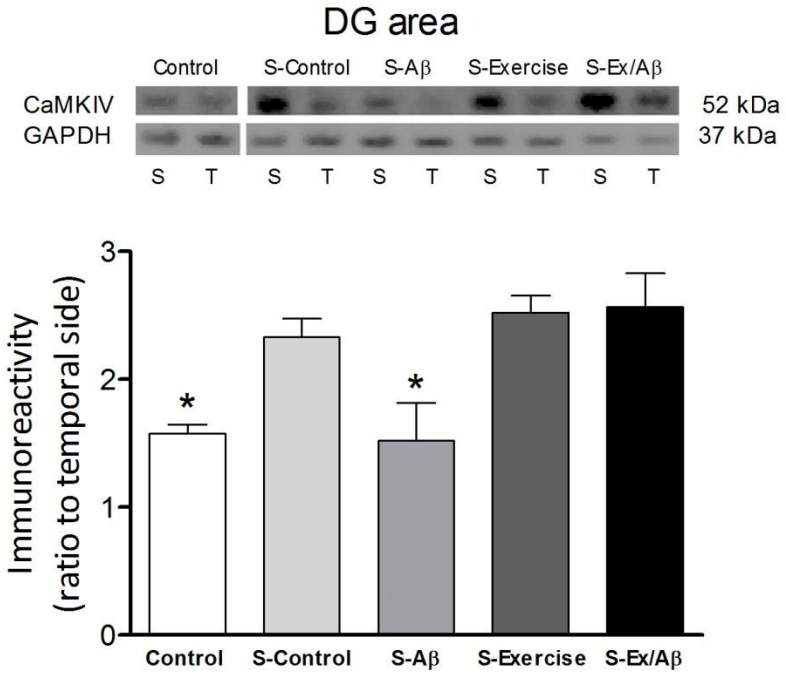

Figure 59. Levels of CaMKIV in DG area after MHFS application. MHFS fails to increase the level of CaMKIV S-Aβ rats but significantly elevates those of S-Control, S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from S-Control, and S-Exercise and S-Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion.

4.3.6.3. Levels of BDNF after L-LTP expression in DG area

Previous data showed that our E-LTP protocol did not increase the level of BDNF in the hippocampus of control rats (Figure 51 and Figure 54). However, MHFS induced L-LTP in DG area successfully produced a robust increase in BDNF levels in the perforant synapses. Five hours after L-LTP induction, we observed significant changes in levels of BDNF in the DG area of S-Control (1.499 ± 0.2836), S-Exercise (1.646 ± 0.5465), and S-Ex/Aβ rats (1.803 ± 0.4495) compared to unstimulated control rats (0.8168 ± 0.2208) ($p= 0.01 - 0.05$) (Figure 60). However, this effect was not seen in Aβ rats (0.8131 ± 0.3337) indicating the possible role of BDNF in mediating the protective effect of exercise against AD-induced impairments.

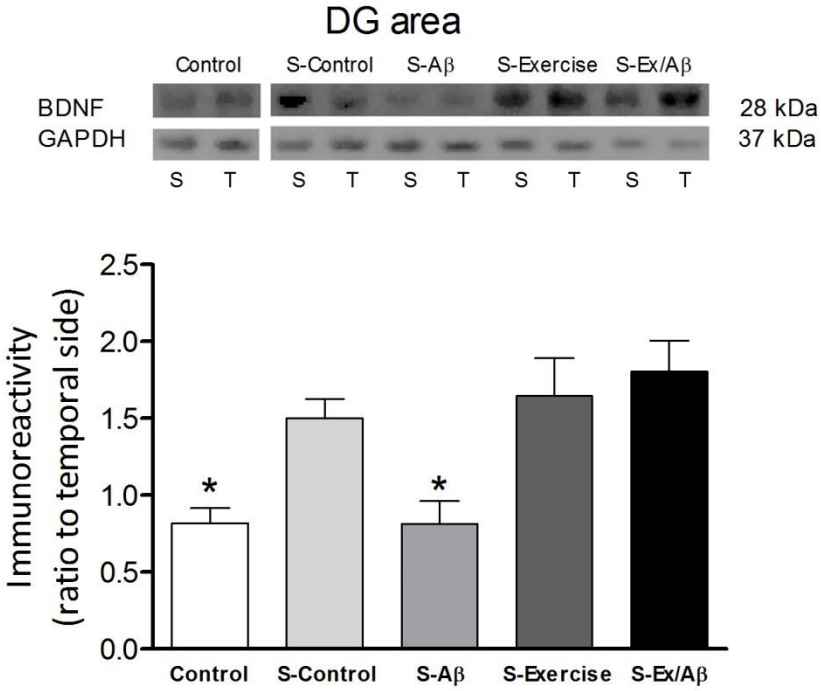

Figure 60. Levels of BDNF in the DG area after L-LTP induction via MHFS. MHFS fails to increase the level of BDNF in S-Aβ rats but significantly elevates those of S-Control, S-Exercise and S-Ex/Aβ rats. (*) indicates significant difference from S-Control, and S-Exercise and S-Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., n = 4-6 rats/group. Insets are representative western blots. S: septal portion, T: temporal portion of the DG area.

5. DISCUSSION

Accumulating evidence suggests that exercise has profound benefits on physical and mental health. Human and animal studies show that regular exercise training plays an important role in correcting cognitive deficits associated with aging and various brain insults. In contrast, Alzheimer's disease is well-documented to progressively affects the integrity of the brain structures involved in learning and memory, which eventually results in irreversible memory loss. However, the impact of exercise on symptoms of AD pathology has yet to be thoroughly tested. Thus in this study, we investigated the effects of 4 weeks of regular moderate treadmill on cognitive and non-cognitive functions in a rat model of AD-like pathology utilizing behavioral, electrophysiological, and molecular approaches. The aim of this project is to evaluate whether exercise could potentially prevent spatial learning and memory and anxiety-like behaviors in this AD rat model. The study also reveals the synaptic changes associated with AD pathology and/or exercise training, which may correlate with signaling cascades deficits involved in the disease pathogenesis.

Previous data from our lab reported that a 1:1 mixture of Aβ proteins ($A\beta_{1-40}$: $A\beta_{1-42}$, 300 pmol/day, 2 weeks) produced spatial learning and memory deficits accompanied by basal synaptic transmission impairment, E-LTP and L-LTP inhibition, LTD facilitation and deleterious changes in key signaling pathways (Srivareerat et al., 2009, Alkadhi et al., 2011). In the present study, we utilized the same model with a full dose of amyloidogenic $A\beta_{1-42}$ (250 pmol/day, 2 weeks) as $A\beta_{1-42}$ peptide is believed to be extremely prone to accumulation forming the major component of senile plaques

(Harigaya et al., 2000). Our behavioral, electrophysiological, and molecular data consistently confirm that 4 weeks of moderate treadmill exercise prevents the detrimental effect of amyloid infusion on the brain. In particular, regular exercise prevented spatial learning, short-term and long-term memory deficits as shown by results in the RAWM task. Additionally, basal synaptic transmission impairment and E-LTP and L-LTP suppression associated with Aβ pathology were alleviated by prior regular exercise. In correlation with behavioral and electrophysiological findings, molecular analyses revealed that Aβ-induced deleterious changes in levels of synaptic plasticity-related molecules (CaMKII, PP2B, CaMKIV, CREB, BDNF) were absent in Aβ animals that had been exercised for 4 weeks. Furthermore, failure of repetitive stimulation to increase the levels of p-CaMKII, p-CREB, CaMKIV, BDNF in the CA1 and DG areas of Aβ rats is prevented by our exercise regimen. Perhaps, by restoring kinase-phosphatase balance with an increase in BDNF availability, regular exercise prevents impairment of basal synaptic transmission and LTP induced by amyloid infusion, which eventually translates to normal behaviors as seen in the RAWM and anxiety-like behavior tests.

5.1. Alzheimer's disease-like pathology rat model:

Alzheimer's disease remains incurable partly due to the lack of animal models that can represent the full-blown symptoms observed in humans. Nevertheless, animal research has been successful in reproducing neuropathological hallmark (i.e. amyloid plaques) and cognitive impairments in both transgenic and non-transgenic models. Most transgenic mice express mutations in one or all of APP, PS1, and PS2 genes, which result in deficient learning and memory with evidence of amyloid deposits but not of fibrillary

tangles formation and neuronal death (Irizarry et al., 1997, Higgins and Jacobsen, 2003). Additionally, genetic component of AD accounts mainly for the early onset type of the disease (familial AD), which is less than 5% of all AD cases (Alzheimer's disease Association). The rat models of AD, including exogenous administration of amyloid peptides directly into certain brain areas, provide an alternative to those transgenic models.

We utilized the AD model generated by continuous i.c.v. infusion of $A\beta_{1-42}$ at a dose of 250 pmol/day for two weeks in rats that may represent the sporadic type of AD (Nitta et al., 1994, Itoh et al., 1996, Nakamura et al., 2001, Srivareerat et al., 2009, Alkadhi et al., 2011). Studies from this lab have shown that a concentration of 160 pmol/day of $A\beta_{1-42}$ does not produce AD symptoms (Tran et al., 2010) while a concentration of 300 pmol/day of $A\beta_{1-40}$: $A\beta_{1-42}$ in a 1:1 ratio reproduces AD symptoms such as cognitive impairment and synaptic plasticity deficits (Srivareerat et al., 2009). These findings suggest that there is a threshold, which marks the maximal ability of neurons to withstand the insults. The advantages of using exogenous administration of amyloid peptides include: 1) Intracerebral ventricular (i.c.v.) infusion of amyloid peptide models the sporadic type of AD, which accounts for more than 95% of AD cases, 2) The ability to infuse a specific length peptide with a predetermined amount and duration allows the identification as well as revealing the role of that specific peptide in AD pathogenesis (Stephan and Phillips, 2005), 3) Experiments can be accelerated rather than waiting for the animal to age, 4) The rat brain is more extensively studied compared to that of the mouse (Martinez and Kesner, 1998), and 5) Bigger rat brain

allows easier manipulation, especially with *in vivo* electrophysiological recordings. Additionally, the group housing is cost and space effective. Nevertheless, the i.c.v. infusion of amyloid in rat as an AD model also has its own disadvantage as it is an invasive procedure, which may cause unexpected inflammation and injuries at the infusion site. Also, the vehicle that carries the peptide may produce adverse effects. However, these obstacles can be overcome by adjusting the infusion rate, better hygiene, and choosing the appropriate vehicle.

5.2. Moderate treadmill exercise protocol:

Current consensus views the effect of exercise on cognitive function as an inverted U-shape in which too much or too little exercise does not benefit the brain health (Kamijo et al., 2004). In accessing the benefits of exercise, our lab has used a forced treadmill running protocol at moderate duration and intensity. The treadmill system delivers a mild electric shock (~ 0.5 mA) if the rat stops running. The electrical shock is strong enough to encourage the animals to run but not intense enough to cause significant stress to the animals (Vollert et al., 2011). The influence of environmental stress was also eliminated by placing the sedentary animals on the treadmill without running for the same period of exercise training time. This particular forced exercise regimen was utilized for two main reasons. Unlike wheel running, animals were forced to run a predetermined distance facilitating quantification and extrapolation into human exercise regimens. Furthermore, animals run with cagemates, which avoids isolation stress inherent to singly housed animals using voluntary running wheels. It is noteworthy that our behavioral, electrophysiological, and molecular findings

consistently indicated that the exercise regimen used in this study did not enhance learning and memory in cognitively normal rats while it is neuroprotective in the context of infusing amyloidogenic peptides.

5.3. The behavioral effects:

Due to the multi-factorial nature of the disease, so far AD remains incurable. Extensive evidence suggests the benefits of exercise on both cognitive and non-cognitive functions. In fact, clinical studies of residents of nursing home diagnosed with dementia associated with neurodegenerative disorders (e.g. Alzheimer's disease) have shown promising results with exercise as a potential treatment of these incurable diseases (Venturelli et al., 2011, Cheng et al., 2012a, McLaren et al., 2013). These AD patients exhibited a better memory performance in cognitive testing tasks after regular exercise training (Venturelli et al., 2011). In addition, these clinical studies have shown several advantages in using regular exercise as a possible AD treatment. For example, regular exercise is a non-pharmacological approach, which is inexpensive with no major adverse effects. Additionally, exercise allows flexibility where the individuals can decide to exercise at their own leisure time.

5.3.1. Learning and memory – the water maze paradigm

Spatial learning and memory can be tested in animals using various maze models. Among these, radial arm water maze (RAWM) is considered to be an effective tool that allows quantification of learning and memory. RAWM is a hybrid of Morris water maze (MWM) and radial arm maze (RAM), which utilizes the advantages of both paradigms while eliminating their drawbacks (Diamond et al., 1999, Shukitt-Hale et al.,

2004, Alamed et al., 2006). Unlike the MWM where the animal may swim around the walls or reluctantly float until hitting the platform, the design of the RAWM forces the animals to navigate through the arms or the opened center area of the pool and presumably using spatial cues. The complexity of the task in the RAWM requires the animals use their ability of spatial learning and memory instead of non-stoppable swimming effort in search for the hidden platform. Additionally, overtraining confounding effects seen with MWM can be completely abolished with the RAWM procedure as the animal go through only 12 learning trials, one short-term memory, and one long-term memory test with resting time in between. Previous studies have also reported suitability of the RAWM in testing spatial learning and memory in models of acute sleep deprivation (Zagaar et al., 2012), psychosocial stress (Tran et al., 2010), and high-fat high-carbohydrate diet (Alzoubi et al., 2013).

5.3.2. Alzheimer's disease and memory

The present study utilized a rat model of AD-like pathology generated by continuous i.c.v. infusion of $A\beta_{1-42}$ (250 pmol/day, 2 weeks) to mimic the progressive accumulation of amyloid peptides seen in the sporadic type of AD. In agreement with previous findings (Nitta et al., 1994, Nitta et al., 1997, Srivareerat et al., 2009), amyloid-infused (Aβ) rats showed impairment of spatial learning, short-term and long-term memory as indicated by poor performance in the RAWM compared to controls. During behavioral assessment, we also observed that the Aβ rats spent significantly more time in the central area of the pool appearing disoriented. Unlike other rats, the amyloid-infused rats were reluctant to swim into the arms and failed to navigate using their tails.

After being guided manually toward the platform at the end of each learning trial, these rats simply sat on the platform and without any interest in visually exploring the surroundings, which was not observed in rats of the other groups. This observation leads us to several thoughts: 1) amyloid burden may interfere with synaptic connections that require encoding and associating new information, 2) the toxic peptide infusion results in lack of self-motivation with possible impairments of locomotor activity as revealed by OF and EPM paradigms. Our behavioral findings are consistent with other AD studies in which transgenic mice carrying AD-related genes mutations or animals with lesions in the hippocampus, basal forebrain, and nucleus of Meynert displayed both spatial and non-spatial learning and memory as shown in various behavioral paradigms (Isomae et al., 2003, Nichol et al., 2007, Faraji et al., 2008, Hoveida et al., 2011).

During the first set of learning trials, similar performance was seen across all groups as the animals started to learn the task. As the learning trials continued, there was a clear difference between Aβ rats and other groups (Figure 22A). Also, Aβ rats lost the ability to retain the learned information inasmuch as during the short-term memory test, 30 minutes after the last learning trials, these rats seemed unable to remember the task; and thus, scored with higher number of errors compared to all other groups. Similar trend was observed in the long-term memory test, given 24 hrs after the last learning trial, Aβ rats performed poorly in the RAWM and they behaved like "first-timers" being in the maze. Thus, it seems that continuous infusions of toxic $A\beta_{1-42}$ affects

the integrity of the hippocampus, which manifests as impairment in spatial learning and memory task.

Investigations regarding the behavioral effects of exogenous administration of various amyloid species have been varied. Some studies reported impairment in both short-term and long-term memory (Nakamura et al., 2001, Srivareerat et al., 2009), and objection recognition (Nag et al., 2001) while others showed that only short-term memory was impaired (Stepanichev et al., 2003). Perhaps, this difference results from the selection of peptide length, treatment duration, or brain regions targeted (Stephan and Phillips, 2005). With this in mind, investigators should carefully decide the choice of peptide, techniques employed in order to make the correct correlation between biochemical changes, stages of the disease, and behavioral manifestations.

Behavioral mazes are designed based on the core assumption that animal performance is the quantification of learning and memory. Hence, there are factors that might interfere with the animal performance besides the effect of certain treatments. For example, other factors such as impaired motor function and anxiety-like phenotypes as well as stress (continuously being dropped in the water) might affect the animal performance without actually altering cognitive function. We observed no visible motor dysfunction as the amyloid-infused rats ran on the treadmill similarly to control exercised rats. Additionally, resting time between two sets of learning trials avoids muscle fatigue associated with swimming and allows familiarization of the animals to the RAWM environment. Also, it is possible that stress contributes to the deficits seen at the cellular and molecular levels. However, it is believed that stress affects the

hippocampus differently than amyloid toxicity. For instance, psychological stress using the intruder model does not disrupt learning acquisition and long-term memory, and L-LTP in the DG area (Gerges et al., 2001, Alzoubi et al., 2005, Tran et al., 2010). Data from this study support the notion that AD pathology destroys spatial learning and memory which eventually manifests as global destructions of synaptic transmission and negative alteration LTP-signaling pathways in both CA1 and DG areas.

5.3.2. Exercise, Alzheimer's disease, and memory

The learning and memory impairment, seen in the rat model of AD in this study, has been shown to reverse two weeks after cessation of the infusion (Nitta et al., 1997). This observation suggests a transient effect of amyloid toxicity on cognitive function. A substantial body of evidence proves that passive and active immunization against toxic amyloid peptides can reverse memory impairment, probably by reducing the amount of amyloid proteins and thus preventing its accumulation and aggregation (Lee et al., 2006, Lemere and Masliah, 2010). Therefore, it is possible that coupling amyloid toxicity with other risk factors such as aging, oxidative stress, and disruption of calcium homeostasis may turn a transient phenomenon into a permanent memory loss, which is often observed in the human AD pathogenesis.

Since it is first described in 1906 by Alois Alzheimer, AD remains incurable with an unknown etiology and multifactorial nature. Non-pharmacologic intervention such as exercise is an interesting prospect in preventing AD incidents and progression as several randomized trials confirm that exercise can improve cognition in people with AD (Lautenschlager et al., 2008, Yu and Kolanowski, 2009). Also, one year of exercise is able

to prevent cognitive decline in nursing home residents with mild to moderate AD more efficiently compared to patients receiving routine medications only (Rolland et al., 2007). Hence, exercise can be a potential prophylactic treatment for those who are at risk of developing AD, and a therapy to ameliorate the cognitive impairment in pre-existing mild and moderate AD.

Similar results have been also reported in exercised animals affected by AD pathology. Studies in transgenic mice reported an improvement of behavioral performance after exercise. In the MWM, mice that ran either on the treadmill or running wheel show decreased escape latencies and swimming distance to the target platform compared with sedentary mice (Um et al., 2008). Additionally, in the MWM task exercised mice or ibotenic-induced lesion (AD model) exercised rats spent a longer time in the correct quadrant where the platform is located compared with their controls (Adlard et al., 2005, Hoveida et al., 2011). Decreasing amyloid load and plaque deposition probably are among the first findings of the beneficial effects of exercise in AD models (Nichol et al., 2009, Adlard et al., 2005, Yuede et al., 2009, Um et al., 2008). In agreement with these studies, our preset findings showed that exercise totally prevented the negative effect of amyloid infusion on spatial learning and memory. The learning curve of Ex/Aβ rats looked similar to those of control and exercise rats throughout the training period (Figure 22A). Moreover, exercise seems to prevent AD-induced inability of retaining information as Ex/Aβ rats performed similar to control rats in the RAWM (Figure 22B, 22C). Interestingly, our exercise regimen does not improve spatial learning and memory in cognitively normal rats, which seems to be in

discrepancy with other studies in which exercised normal animals perform better than sedentary normal controls in behavioral tasks (Drumond et al., 2012, Cetinkaya et al., 2013). Other studies confirm our findings as exercise exerts a neuroprotective rather than neuroenhancing effect on cognitive function (Zagaar et al., 2012). This incongruity maybe due to different exercise training protocols and sensitivity of the behavioral tests employed.

Performance in the RAWM could be confounded by the fact that exercised animals might swim faster and better as they were accustomed to physical training. Even though we did not measure the swim speed of each rat, we did not observe obvious differences in the swimming ability across all groups. Indeed, studies reported that the improved performance of exercised animals is not due to variation in swimming speed but rather to a better remembering of the task (van Praag et al., 1999, Adlard et al., 2005, Um et al., 2008). Altogether, our findings suggest that 4 weeks of treadmill exercise exerts neuroprotective effects on Aβ as seen in behavioral performance.

Exercise does not only improve behavioral performance in spatial learning and memory tasks but also in non-spatial memory paradigms such as object recognition and passive avoidance. Furthermore, in a model of Alzheimer's disease induced by ibotenic acid injection in the nucleus basalis magnocellularis, treadmill exercise significantly improved passive avoidance learning (Hosseini et al., 2013).

5.3.2. Non-cognitive functions

5.3.2.1 Alzheimer's disease and non-cognitive functions

In addition to the progressive memory loss, the clinical population of AD patients also experience a wide array of non-cognitive symptoms ranging from olfactory impairment, gait and balance dysfunction to psychiatric problems (Raudino, 2013). A population-based study reported that AD patients exhibited various psychiatric phenomena including delusion, hallucination, mania, severe depression, agitation, wandering, and apathy (Burns et al., 1990). Furthermore, AD pathology negatively regulate sleep quality as the patients have reverse day-light cycle and increased agitation, which create additional stress burden for their caregivers (Vitiello and Borson, 2001). Often considered as manifestations in later stages of the disease, behavioral and psychological symptoms of AD such as social withdrawal, paranoia, and mood swings actually appeared long before its clinical diagnosis (Jost and Grossberg, 1996). As the pathology progresses, other non-cognitive disturbances (e.g. anxiety, irritability) become more prominent. In fact, the majority of AD population (about 90%) develops behavioral abnormalities, which highly correlate with the degree of cognitive decline (Mega et al., 1996). Neuropsychological symptoms and behavioral disturbances place a heavy psychologically and financial burden on the caregivers of AD patients (Moore et al., 2001). Indeed, these non-cognitive deficits associated with the disease are considered to be the most challenging yet distressing element of AD pathology (Alzheimer's Disease Association).

The non-cognitive disturbances associated with AD are well-documented in humans but have not been fully investigated in animal models of AD. Experimental animals might not exhibit a whole spectrum of psychological behaviors similar to humans. Additionally, appropriate evaluation of these symptoms has been challenged due to the lack of sensitive behavioral testing. Recently, non-transgenic and transgenic studies of AD in mice have reported depression-like behaviors, increased anxiety, and apathy (Filali et al., 2012, Chen et al., 2013). In the current study, we also reported neuropsychiatric symptoms (i.e. anxiety-like behaviors) in a rat model of sporadic AD as shown in a battery of behavioral tests including open field (OF) apparatus, light-dark (LD) box, and elevated plus maze (EPM). Compared to control rats, Aβ rats displayed increased anxiety-like behaviors as they tend to move along the walls of the OF apparatus, and stay longer in the dark compartment of the LD box and the closed arms of the EPM. It seems that amyloid infusion not only exert a toxic effect on the hippocampus, a depository of spatial memory, but also in other brain regions controlling anxiety behaviors. We speculate that an overt amount of exogenous $A\beta_{1\text{-}42}$ peptides will circulate in the cerebral spinal fluid (CSF) to all brain regions, and depending on the vulnerability of specific neurons to chemical insults, certain brain areas accumulate more of amyloid proteins than others; thus increasing the propensity of Aβ peptide aggregation and eventually functional disruption in these brain regions. In the amygdala, a limbic structure known to directly influence fear and anxiety-related behaviors (Shin and Liberzon, 2010), there is prominent atrophy even in the early stage of AD and this limbic atrophy is strictly correlated with the disease severity (Poulin et al., 2011). The

atrophy of the amygdala associated with AD is probably due to a reduction in total number of neurons, especially the medium and large neurons while the small neurons undergo nucleus shrinkage (Scott et al., 1992). Neuropathological studies reveal the presence of neuritic plaques and neurofibrillary tangles in the AD brains, which possibly explain emotional and motivational disturbances in AD patients (Kromer Vogt et al., 1990, Unger et al., 1991).

We also examined the effect of AD-like pathology on locomotor activity in the OF and EPM. Both tests confirmed that amyloid infusion severely impaired locomotor activity as shown by shorter total distance travelled and lesser total activity (Figure 23B, 23C, 25B, 25C). These Aβ rats have the tendency to confine to a certain area with no interest to explore the whole open field or the arms of the EPM. This is likely due to the effect of toxic amyloid on motor neurons by increasing threshold for firing or inducing neuronal death. In our study, the impaired locomotor activity seen in Aβ rats seems not to be the result from motor dysfunction as these rats run well on the treadmill with no noticeable motor deficits but rather from lack of motivation. In contrast to our finding, a study reports increased locomotor activity in triple transgenic mice model of AD, but only detected at 10 months old (Knight et al., 2013). This can perhaps be explained by different approaches used to create the model *per se* (amyloid infusion vs. gene manipulation), which may potentially result in different effects on the same parameter studied.

5.3.2.2. Alzheimer's disease, exercise, and non-cognitive functions

Ample evidence suggests that regular exercise is beneficial for learning and memory impairment caused by sleep deprivation, maternal deprivation stress, addiction, and toxic chemical insults (Vollert et al., 2011, Zagaar et al., 2012, Dimatelis et al., 2013, Lynch et al., 2013, Zagaar et al., 2013). However, the impact of exercise on non-cognitive dysfunctions, associated with AD has not been fully investigated.

Our data revealed that AD-like pathology produced non-cognitive disturbances and these detrimental effects were prevented by 4 weeks of moderate treadmill exercise. The anxiety-like behaviors observed in Aβ rats were totally prevented by prior exercise. The Ex/Aβ rats spend a similar length of time in the center area of both OF apparatus and EPM, the opened arms of EPM, and the light compartment of the LD box compared to those of control and exercise rats. This result indicates the anxiolytic effect of exercise on AD-induced cognitive-disturbance. In line with our findings, a recent study showed that in a transgenic mouse model of AD, voluntary exercise prevented the anxiety-like behavior, lack of exploration, and emotionality (Garcia-Mesa et al., 2012). Nevertheless, the mechanism by which exercise exerts a neuroprotective effect on the AD brains remains unclear. It has been postulated that by reducing the AD-induced oxidative stress burden (i.e. decrease abnormal elevated lipid peroxidation), exercise ameliorates non-cognitive symptoms associated with the disease (Cakir et al., 2010, Garcia-Mesa et al., 2012). It appears paradoxical that exercise can effectively protect the brain by this mechanism while at the same time, the production of reactive oxygen species (ROS) gradually increases during exercise (Radak et al., 2008). Thus, with regular

exercise, we speculate that by pre-conditioning the neurons to withstand initial exercise-induced ROS, exercise progressively entrains the neurons to be more resistant in face of toxicity such as amyloid infusion, probably via BDNF-related mechanism.

In this study, the impact of exercise training and/or $A\beta_{1-42}$ infusion on locomotor activity was also evaluated. Surprisingly, exercise did not prevent locomotor activity impairment in amyloid-infused rats as these Ex/Aβ rats displayed less total activity and total distance travelled in both the OF apparatus and EPM paradigm similar to that of Aβ rats, which is significantly different from controls. Probably our exercise protocol is not strong enough to rescue this AD-induced impairment.

5.4. Electrophysiological investigations

Memory consolidation and retrieval require the integrity of synaptic circuits among neurons across from several brain regions including the hippocampus and neocortex. The efficacy of encoding and storing new information depends heavily on the strength of connections between neurons and its neural network. Long-term potentiation (LTP) is well-recognized as the closest cellular analogue of learning and memory. In various animal models of learning and memory impairments, LTP suppression is also reported. For example, sleep deprivation results in spatial learning and memory deficits, which correlate with hippocampal LTP suppressions (Zagaar et al., 2012, Zagaar et al., 2013). Additionally, reduction or blockage of LTP-related key signaling molecules produces impaired spatial learning and memory performance as well as hippocampal LTP (Bach et al., 1995). In order to investigate the impact of exercise training and/or AD pathology on the hippocampal synapses, we utilized *in vivo*

extracellular recordings in the CA1 and DG areas, which gave us several advantages over the intracellular recording technique. Compared to *in vitro* recording, extracellular recording in live anesthetized animals allows the direct study of pharmacological treatment under physiological conditions and thus more validity and applicability of the results. Moreover, extracellular recording provides accurate information regarding the status of interconnected circuits of neurons, which are implicated in mediating certain behaviors.

5.4.1. Alzheimer's disease and synaptic plasticity

Hippocampal synapses are quite vulnerable in AD pathogenesis and progression. In experimental setting, LTP induced via repetitive stimulations is a valuable tool for evaluating therapeutic treatments for disorders of the central nervous system (CNS). Amyloid oligomers can inhibit LTP both *in vitro* and *in vivo* in various hippocampal areas involved in the learning and memory processes (Huang et al., 2006, Srivareerat et al., 2009, Ma et al., 2011, Srivareerat et al., 2011). Previous findings from this lab have shown that amyloid peptides infusion can impair synaptic plasticity both in the hippocampus and sympathetic ganglia (Srivareerat et al., 2009, Alkadhi et al., 2011, Alzoubi et al., 2011). Experiments in area CA1 cultured neurons indicate that amyloid proteins can induce depression of basal glutamatergic synaptic transmission via both pre- and post- synaptic mechanisms (Yao et al., 2013) and suppress LTP through oxidative damage (Kapay et al., 2013). Transgenic AD mice exhibit learning and memory deficits in addition to impairment of the prefrontal and perirhinal cortex synaptic plasticity (Tamagnini et al., 2012, Lo et al., 2013). Indeed, clinical studies in AD patients

demonstrate an impaired LTP-like response in cortical areas (Koch et al., 2012) and this synaptic dysfunction was also observed in other brain regions (e.g. hippocampus) in animal models of AD (Nalbantoglu et al., 1997, Chapman et al., 1999, Stephan et al., 2001, Srivareerat et al., 2009). Consistent with these findings, we found that 2 weeks infusion of $A\beta_{1-42}$ shifted the input/out (I/O) curves of Aβ rats to the right, which is indicative of impaired basal synaptic transmissions. Under the effect of amyloid infusion, Schaffer collaterals and perforant synapses require much higher voltage in order to elicit the same response as from controls. We also observe suppression of E-LTP and L-LTP in these synapses, which highly correlates with behavioral findings. Other studies also demonstrate severe impairments of early and late phase LTP in hippocampal slices of transgenic AD mice compared to wild-type controls (Dominguez-del-Toro et al., 2004, Auffret et al., 2010, Chong et al., 2011). The mechanism of AD-induced inhibitions of basal synaptic transmission and LTP remains unclear. However, it has been postulated that cellular ionic dysregulation may contribute to AD pathology by embedding amyloid oligomers into the membrane to form amyloid channels (Capone R, 2012a, b, Connelly et al., 2012), which in turn directly or indirectly alter ion fluxes and membrane ion channel expression and activity (Calon et al., 2005, Dewachter et al., 2009).

In addition to fEPSP slope measurement, we also evaluated the pspike amplitude, which represents the number of neurons that reach threshold and fire in this AD rat model. The CA1 pyramidal cells displayed significantly lower pspike amplitude upon both E-LTP and L-LTP induction compared to all other groups indicating that

amyloid accumulation probably interferes with the synapses raising the threshold for neuronal firing. To our surprise, the pspike amplitude in the DG area of Aβ rats remained unaltered after HFS. It seems that the resilience and different properties of granule cells of the DG compared to the pyramidal cells of CA1 area may contribute to this interesting effect of amyloid toxicity. This is discussed further in section 5.4.4.

At the molecular level, the inhibitory effect of AD pathology on the hippocampal synapses is due to deleterious alterations in levels of LTP-related signaling molecules. For example, we found a significant reduction in levels of p-CaMKII with an aberrant increase in levels of calcineurin in amyloid-infused rats under the basal and E-LTP induction conditions in both CA1 and DG areas. Similarly, molecules involved in long-term changes at the synapse including p-CREB and CaMKIV were also negatively affected in Aβ rats. Further discussion on the impact of exercise and AD pathology will follow in section 5.5.

5.4.2. Exercise and synaptic plasticity

Synaptic plasticity is an activity-driven change in the strength of the synapse which can exist in two forms: long-term potentiation (LTP) and long-term depression (LTD). While LTD is unaffected by exercise (Christie et al., 2008), LTP is considered to be positively altered during exercise training. For instance, LTD expression is not significantly different between exercise and control animals using the same stimulation protocol (Vasuta et al., 2007).

In contrast, treadmill running increases LTP expression as seen through increases in field excitatory post-synaptic potential (fEPSP) slope and population spike (pspike)

amplitude in the DG both *in vivo* and *in vitro* (Farmer et al., 2004, O'Callaghan et al., 2007). One of the proposed mechanisms attributed to this beneficial effect is the ability of exercise to lower the threshold for LTP induction leading to increased neuronal firing and eventually resulting in enhanced LTP magnitude (Farmer et al., 2004). Additionally, exercise can also reverse LTP deficits in diabetic rats suffering from learning and memory impairment (Reisi et al., 2010). Our exercise regimen *per se* does not seem to alter basal synaptic transmission or LTP magnitude as exercise animals exhibit similar synaptic plasticity phenomena compared to those of controls. Neither the I/O curve nor fEPSP slopes during E-LTP and L-LTP induction in CA1 and DG areas were affected in cognitively normal rats by exercise training. The present findings seem to be in disagreement with other aforementioned studies, which could be explained by differences in exercise duration, intensity, and paradigms. Even though it is not significant, there is a trend showing that exercise seems to lower the voltage in normal rats to produce minimal and maximal responses, and this effect is more prominent in the perforant synapses (Figure 28B, 29B).

In addition to the enhancement of LTP, exercise can regulate the expression of other molecules that are critical for synaptic plasticity including synapsin 1 and CREB. The levels of synapsin 1, a protein that participates in vesicle pool formation and neurotransmitter release, and CREB, a transcription factor that regulates expression of the BDNF gene, positively correlate with the duration of exercise training (Ying et al., 2005, Vaynman et al., 2006b). In fact, the favorable effects of exercise on synaptic

plasticity are largely though to be mediated through BDNF signaling (Vaynman et al., 2003, Kramar et al., 2004, Vaynman et al., 2004, Vaynman et al., 2006a).

5.4.3. Alzheimer's disease, exercise and synaptic plasticity

In this study, we investigated the effects of treadmill exercise and/or amyloid infusion on basal synaptic transmission, long-term potentiation (LTP) in the CA1 and DG sub-regions of the hippocampus. LTP in these two regions can be evoked by multiple trains of HFS and strictly depend on NMDA receptors. Given the opposite effects that exercise and AD pathology have on synaptic plasticity, it is of great interest to investigate how pre-conditioning the pathological brains with exercise training could possibly prevent hippocampal synapses from being negatively altered by the disease pathogenesis and progression.

At the synaptic level, our data indicated that 4 weeks of moderate treadmill exercise protected the perforant and Schaffer collaterals synapses against the deleterious effect of AD pathology. For example, the right side shift of the I/O curve observed in the CA1 and DG areas of Aβ rats was completely absent in animals with exercise training as the I/O curve of Ex/Aβ rats was similar to those of control rats. Furthermore, the voltages required to elicit minimal and maximal response in Ex/Aβ and control rats were not different. Perhaps by increasing synaptic excitability and fostering synaptic connections between neurons, exercise prepares the hippocampal synapses to withstand amyloid toxicity and prime these synapses for activity-driven changes (i.e. LTP). Indeed, we found that CA1 pyramidal cells of Aβ rats exhibited markedly lower fEPSP slope and pspike amplitude throughout the E- and L-LTP recording periods while

prior exercise abolished this negative effect (Figure 28). The HFS-evoked pspike amplitudes of Ex/Aβ rats in area CA1 seem to be lower compared to those of controls but significantly higher than those of Aβ rats. This interesting result signifies the partial effect of exercise in rescuing amyloid-infused pyramidal neurons. Unlike the cells in area CA1, DG granule neurons of Aβ rats showed lower fEPSP slope but normal pspike amplitude during LTP compared to control rats and this decrease in fEPSP slope of amyloid-infused rats was totally prevented by treadmill exercise.

5.4.4. Differences between the CA1 and DG areas

Even though the literature extensively discusses the role of the whole hippocampus in learning and memory process, the function of each sub-region is not adequately investigated. The unidirectional pathway of the hippocampal trisynaptic circuit allows extensive connections between the CA1 and DG areas. These two sub-regions share the same laminar arrangement and have NMDA dependent LTP mechanisms. However, the CA1 and DG areas are quite different in term of cell type population, resilience to insults, distributions and regulations of neurotransmitters and receptors. For example, in hippocampal rat slices, noradrenaline modulates the release of glutamate via presynaptic β-adrenergic receptors in the dentate gyrus but not in area CA1/CA3 (Lynch and Bliss, 1986). Furthermore, CA1 pyramidal cells but not DG granule cells express a delayed rectifying inward current, which leads to difference in discharge behaviors (Stabel et al., 1992). Application of tetraethylammonium (TEA) induces LTD in the dentate gyrus but potentiates LTP in area CA1 suggesting differential drug effect on each sub-region (Song et al., 2001). These differential responses are attributed to the

higher density of T-type calcium channels of the CA1 area compared to the DG (Song et al., 2002) and regional difference of GABAergic modulation between these hippocampal areas (Suzuki and Okada, 2007). In addition, area CA1 and DG also display different functional roles in spatial learning (Okada et al., 2003). Most importantly, compared to the CA1 pyramidal cells, the granule cells of DG area are quite resilient in various insults such as chronic stress, hypothyroidism, anoxia, obesity, and transient cerebral ischemia (Hsu et al., 1998, Yao et al., 1998, Gerges and Alkadhi, 2004, Alzoubi et al., 2005). The resistant property of DG neurons is probably due to different kinase-phosphatase profile compared to that of CA1 neurons (Gerges et al., 2005) and a stronger expression of calbindin-positive interneurons in DG area compared to those of area CA1 (Snyder, 2010). Nevertheless, our data indicates that our AD rat model severely impaired basal synaptic transmission and synaptic strength during E-LTP and L-LTP in both CA1 and DG areas.

Another prime feature of the DG is neurogenesis, which takes place under both physiological and pathological conditions (Sawada and Sawamoto, 2013). Thus, the DG houses various neurotrophic factors including BDNF (Lee and Son, 2009). BDNF plays an important role in growth, differentiation, and survival of neurons, which helps to protect the brain from various insults. It is not coincidental that the levels of hippocampal BDNF are highly up-regulated during exercise as found in our study and others (Cassilhas et al., 2012, Bechara and Kelly, 2013, Dao et al., 2013). Not only exercise elevates the levels of BDNF in the brain but also in the peripheral system even with pre-existing pathologies (Coelho et al., 2013). Even though we did not measure DG neurogenesis, we speculate

that infusion of Aβ protein in our AD rat model can stimulate neurogenesis as an initial compensatory reaction against brain insult caused by continuous infusion of amyloid peptides, which partly explains the finding we observed with the pspike amplitude measurements during E- and L-LTP in the DG area. The pspike amplitude accounts for all neurons including newborn neurons while the fEPSP slope is only a representation of existing neurons with functional synapses in the circuit. Hence, we reported a low fEPSP slope but normal pspike amplitude in the DG area of Aβ rats.

5.5. The molecular effects of exercise on the brain

AD is a multi-factorial disease with various risk factors, which complicates the process of understanding AD pathogenesis and designing potential treatment. It has been suggested that if the disease onset can be delayed by only one year, the number of AD cases globally can be reduced by 9.2 million (Brookmeyer et al., 2007). In order to understand how exercise can exert a beneficial effect on the brain, we also undertook molecular analysis, which is necessary to identify targets that could serve as potential treatments of AD.

5.5.1. AD-related molecules:

5.5.1.1. APP:

Currently, all AD theories fail to explain the full spectrum of AD phenotypes in humans. Presence of plaques and tangles are often detectable only during the late stage of AD, which makes it hard to pinpoint the exact cause and timing of the disease. Recent experiments have investigated certain factors (e.g. diet, hormones, life-style) that influence AD onset and its progression. Our behavioral and electrophysiological data

consistently revealed that 2 weeks of $A\beta_{1-42}$ infusion severely impaired spatial learning and memory, produced anxiety-like behaviors in addition to inhibition of LTP in hippocampal synapses. These detrimental effects could be the consequence of abnormal regulation in levels of AD-related molecules such as APP.

We found a significant increase in APP levels in both CA1 and DG areas of amyloid-infused rats compared to all other groups. It seems unconventional that increasing the end products of APP (i.e. exogenous administration of amyloid peptides) actually elevates the levels of APP. However, it is noteworthy that infusing toxic $A\beta_{1-42}$ might result in neuro-inflammation, which triggers the production of APP as an anti-inflammatory mechanism via its soluble ectodomain (Allinson et al., 2003). In agreement with our study, reports have shown that in a mouse model of AD, glucocorticoids treatment enhance the production of Aβ *in vitro* and *in vivo* possibly by increasing the levels of both APP and APP cleaving enzyme (Green et al., 2006). The glucocorticoid treatment-induced increase in Aβ is probably mediated by transcriptional activation of APP as glucocorticoid-response elements are found on a sequence of APP promoter (Lahiri, 2004). In the present study, we also evaluated whether treadmill exercise could alter AD-induced increase in the basal levels of APP in CA1 and DG sub-regions. Western blot analysis indicated the abnormal elevation in basal levels of APP was not seen in Ex/Aβ rats. Exercise seems to be able to limit the availability of precursor materials for Aβ synthesis, which in turn may eliminate the toxic effects of induced endogenous and consequently exogenous Aβ on cognitive and non-cognitive functions by curtailing suppression of the hippocampal synapses.

5.5.1.2. BACE-1:

The processing of amyloid precursor protein (APP) is often regulated by a family of secretases. Among these, BACE-1, which is most active in neurons and neuronal tissues (Seubert et al., 1993), is an essential molecule in generating toxic amyloid peptides. BACE-1 knockout mice show no neuronal Aβ production and AD-related pathologies but increased death after birth, lighter weight, and hyperactivity (Dominguez et al., 2005). The BACE-1 role in amyloid production is thought to be modulated via GSK-3 signaling pathway as GSK-3 specific inhibitor abolishes BACE-1-induced APP processing and Aβ production by negatively affecting transcriptional and translational control of BACE-1, thus preventing AD phenotypes (Ly et al., 2013). Overexpression of BACE-1 can substantially increase the production of Aβ (Bodendorf et al., 2002), but combined with APP mutation it produces neuropathological hallmarks such as amyloid deposits and activated astrocytes (Mohajeri et al., 2004). Together, these results suggest an important role of BACE-1 in amyloid proteins biosynthesis.

Various studies report elevated BACE-1 levels in the brains of both familial and sporadic AD (Vassar et al., 1999, Holsinger et al., 2002, Tyler et al., 2002). In line with these findings, we observed a significant increase in levels of BACE-1 in Aβ rats in CA1 and DG areas compared to all other groups. Because BACE-1 promoter contains binding sites of several transcription factors (e.g. NFκB) (Sambamurti et al., 2004), it is likely that altered BACE-1 expression is due to changes in activation of these transcription factors. Indeed, it has been shown that physiological level of endogenous Aβ does not transactivate BACE-1 promoter whereas overt Aβ production results in BACE-1 promoter

transactivation, which depends on NFκB signaling (Buggia-Prevot et al., 2008). Thus, we speculate that in our study, exogenous administration of $A\beta_{1-42}$ results in the production of inflammatory cytokines, which in turn enables NFκB to positively regulate the basal levels of BACE-1 in the brain, which explains the increased basal levels of BACE-1 in Aβ rats. Other studies also report increased mRNA, and protein expression as well as increased activity of BACE-1 in AD patients (Coulson et al., 2010, Ewers et al., 2011).

The present study also revealed that 4 weeks of treadmill exercise prevented AD-induced increase in basal levels of BACE-1 in both CA1 and DG areas, possibly by reducing the levels of BACE-1, a rate-limiting step in the production of toxic Aβ peptides. In amyloid-infused rats, exercise shifts the processing of APP toward a non-amyloidogenic pathway in which shorter length of soluble Aβ proteins are formed and thus decreasing propensity to aggregate.

5.5.2. The crosstalk between the CNS and PNS:

How does muscle movement initiate these protective effects in the CNS? This is an intriguing question that has, as yet, no definitive answer, even though evidence for "cross-talk" between the skeletal muscles and CNS exists. For example, as running velocity increases, the discharge frequency of hippocampal CA1 pyramidal cells and interneurons increases (Czurko et al., 1999). Studies propose that muscle can secrete numerous humoral factors that exert a protective effect on the brain (Pedersen et al., 2007, Pedersen, 2011). Even though the exact molecular mechanism responsible for the cross-talk between the skeletal muscles and CNS remains to be elucidated, experimental data support two possible mechanisms. *First,* events associated with energy balance

play an important role in CNS function. For example, exercise up-regulates hippocampal expression of the mitochondrial molecule, uncoupling protein 2 (UCP2), which in turn protects neuronal mitochondria from oxidative stress, enhances ATP production, and regulates normal calcium level (Vaynman et al., 2006a). In addition, UCP2 can also modulate BDNF signaling and its downstream mediators such as CREB and CaMKII (Vaynman et al., 2003). *Second*, interleukin-6 (IL-6), an immunomodulatory cytokine may be responsible for the crosstalk between the periphery and CNS (see review by Alkadhi, 2012). During exercise training, IL-6 increase is linked to reduced level of glycogen (Steensberg et al., 2001), which in turn can positively regulate glucose homeostasis in the brain.

5.5.3. Signal transduction pathways

There are two different phases of NMDA-dependent LTP in the perforant and Schaffer collaterals synapses. Early phase (E-) LTP, lasting up to 3 hours, is evoked by a single train of HFS and depends on levels of key molecules such as CaMKII and calcineurin. Meanwhile, later phase (L-) LTP lasts up to days and requires protein synthesis with the involvement of CREB, CaMKIV, ERK1/2, and BDNF. In this study, we evaluated the effect of exercise and/or AD pathology on protein expression of these LTP-related signaling molecules in the CA1 and DG areas in order to correlate with behavioral and electrophysiological observations.

5.5.3.1. CaMKII

What are the possible mechanisms by which exercise would be protective of brain damage caused by AD pathology? One of the potential candidates is probably

preservation of molecules that exert effects on both the peripheral and central nervous system; cardinal among them is CaMKII, particularly the subtype α. Even though α-CaMKII is the most abundant protein found in the brain, it is also present in skeletal muscle with a seemingly non-functional kinase role (Chin, 2004). Additionally, it is well-documented that α-CaMKII plays a key role in the learning and memory processes as well as long-term potentiation (LTP) induction and persistency (Sanhueza and Lisman, 2013). For example, genetic knockouts of the α-CaMKII gene result in cognitive deficits and LTP blockage (Silva et al., 1992, Stevens et al., 1994, Matsuo et al., 2009). Moreover, mice that are heterozygous for a null mutation of α-CaMKII exhibited both memory impairment and mood change-like behavior (Yamasaki et al., 2008). In our study, we reported a significant reduction in basal levels of p-CaMKII in CA1 and DG areas of Aβ rats while this alteration was not seen in Ex/Aβ rats. In agreement with our finding, other studies indicate that regular exercise increases expression of hippocampal CaMKII mRNA in rats (Egan et al., 2010) and CaMKII expression and activity in human skeletal muscles (Rose et al., 2007). These exercise-induced molecular effects seem to translate into the ability of exercise to prevent learning and memory deficits and anxiety-like behaviors caused by AD. Interestingly, overexpressing of α-CaMKII in the mouse forebrain produces increased anxiety-like behaviors coupled with offensive aggression (Hasegawa et al., 2009). This discrepancy may be explained by the fact that the brain functions in harmony in which too much or too little is considered to be detrimental. In this study, we also found that the total pool of CaMKII proteins remained unchanged across all groups regardless of exercise or amyloid treatments.

Additionally, studies reported during E-LTP induction the level of p-CaMKII is reduced due to AD-induced impairment of CaMKII phosphorylation (Zhao et al., 2004, Srivareerat et al., 2009, Alzoubi et al., 2011). In line with these studies, our data revealed that HFS resulted in an increase in the levels of both phosphorylated and total CaMKII in normal rats but failed to appreciably increase the level of p-CaMKII in Aβ rats in both CA1 and DG areas. Furthermore, we reported the presence of HFS-induced increase levels of p-CaMKII in these two brain areas in Ex/Aβ rats, which is not different from those of control and exercise rats. Again, the levels of t-CaMKII were similarly elevated upon HFS across all groups in both regions; thus leading to a significantly lower p-CaMKII : t-CaMKII ratio in pyramidal and granule cells of S-Aβ rats but a normal p-CaMKII : t-CaMKII ratio in S-Ex/Aβ rats. Together, these findings indicate that the disease mainly targets the phosphorylation process enabled by CaMKII, which is a critical step for LTP induction.

5.5.3.2. Calcineurin

In addition to LTP inhibition, amyloid exposure can result in up-regulation of phosphatases that regulate CaMKII activation (Chen et al., 2002, Calon et al., 2005, Knobloch et al., 2007, Jo J, 2011). Calcineurin (PP2B) is considered a gating mechanism of LTP as it inactivates kinases and NMDA receptors, thus decreasing channel activity (Lieberman and Mody, 1994, Tong et al., 1995). AD pathology negatively regulates the protein expression of p-CaMKII probably due to the enhanced PP2B levels. Indeed, we found that amyloid-infused CA1 and DG neurons exhibited high basal levels of PP2B compared to all groups. Other studies reported Aβ induces calcium overload and

dysregulation, which eventually manifest as structural and functional disruption of the neuronal networks involved in learning and memory through a calcineurin pathway. PP2B hyperactivity can also result in inflammation, which is commonly observed in the AD brains (Reese and Taglialatela, 2011). We also observed the aberrant increase in basal levels of PP2B in Aβ rats, which were totally normalized by exercise training. Perhaps, by driving the APP processing toward the non-amyloidogenic pathway, exercise reduces amyloid production, which in turn prevents intracellular calcium perturbation via PP2B.

Interestingly, PP2B hyperactivity can also turn on genes transcription that is strictly relate to the late phase of LTP. For instance, oligomeric Aβ reduced p-CREB expression and activity via PP2B-dependent mechanism as inhibition of PP2B normalizes Aβ-induced reduction of p-CREB in the brain. Although the present study did not examine PP2B activity, it is believed that by raising the basal levels of PP2B protein, amyloid infusion can severely impair CaMKII phosphorylation and thus leading to disruptions of cognition and LTP.

In normal rats, HFS is expected to increase the level of PP2B, which is thought to prevent LTP saturation and allow continuous learning. Our data showed that E-LTP induction significantly elevated the levels of PP2B in stimulated control and Aβ rats in both CA1 and DG areas but did not affect those of exercised rats. The inability of HFS to increase PP2B levels in exercised rats, including the Ex/Aβ rats, implies the role of phosphatase in intracellular signaling of learning and memory process, possibly through a BDNF-dependent mechanism or by restoring normal phosphatase-kinase balance.

5.5.3.3. CREB

CREB is an important transcription factor that is responsible for local protein synthesis in order to produce long-lasting changes at the synapse (Lonze and Ginty, 2002). AD pathology negatively affects the protein expression of CREB as shown in isolated hippocampal neurons of transgenic AD mice, cultured neurons exposed to amyloid peptides, and AD post-mortem brains (Pugazhenthi et al., 2011, Ljungberg et al., 2012); thus leading to reduction of downstream molecules, including neuronal glucose transporter 3 (GLUT3), and resulting in decreased glucose uptake and metabolism in the AD brains (Jin et al., 2013). In line with these findings, we observed a significant reduction of p-CREB levels in both CA1 and DG areas of Aβ rats under basal and L-LTP induction conditions. One possible explanation of AD-induced decrease in p-CREB levels is negative modulation by the disease of CREB upstream regulators such as ERK1/2 and CaMKIV. In contrast, regular exercise seems to prevent the deleterious alterations of the CREB/CaMKIV pathway associated with AD pathology. For example, the basal levels of p-CREB and CaMKIV in Ex/Aβ rats were similar to those of control and exercise rats. Additionally, MHFS failed to induce a robust increase of p-CREB in Aβ rat and this failure was prevented by exercise in both CA1 and DG areas. Neither exercise training nor amyloid infusion altered the basal levels of t-CREB in pyramidal and granule neurons suggesting a functional impairment of CREB (i.e. phosphorylation) rather than its protein expression. In addition, our data indicated that MHFS induced a robust increase of t-CREB in all groups including S-Aβ rats compared to unstimulated controls leading to a lower p-CREB : t-CREB ratio in S- Aβ rats and this ratio is normalized in S-

Ex/Aβ rats. In fact, CREB plays an important role in mediating the beneficial effects of exercise-induced upregulation of BDNF, which eventually manifest as better memory performance in exercised animals (Vaynman et al., 2004, Chen and Russo-Neustadt, 2009).

5.5.3.4. ERK1/2 and CaMKIV

ERK1/2 and CaMKIV are known to strictly participate in long-term memory consolidation and L-LTP by their ability to activate CREB via phosphorylation (Lonze and Ginty, 2002). Mice carrying CaMKIV mutation display impaired long-term memory and L-LTP in addition to attenuated CREB phosphorylation but intact E-LTP, learning acquisition, and short-term memory (Kang et al., 2001). Parallel with the reduction in the basal levels of p-CREB in CA1 and DG neurons, we reported a decrease in basal levels of CaMKIV in these two hippocampal areas of Aβ rats. Surprisingly, we found no changes in the basal levels of phosphorylated and total ERK1/2 in both hippocampal sub-regions across all groups; thus CaMKIV maybe a main kinase for activation of CREB. Recent study also reported a reduced protein expression of CaMKIV in a double transgenic mice model of AD (Arrazola et al., 2009) suggesting that by limiting CaMKIV availability, amyloid infusion reduces CREB activation, hence suppresses L-LTP and impairs spatial long-term memory. The fact that 4 weeks of treadmill exercise can prevent AD-induced down-regulation of CaMKIV basal levels indicates that exercise can maintain a normal CREB phosphorylation and intact L-LTP under the insults of $A\beta_{1-42}$ toxicity.

Similar trend was observed in both CA1 and DG areas after L-LTP induction. For instance, MHFS failed to increase appreciably the levels of CaMKIV in S-Aβ rats but not in those of control and exercised rats including Ex/Aβ rats compared to unstimulated controls. The activity-dependent expression of CaMKIV in the hippocampus of exercised rats suggests that increasing CaMKIV availability will enhance CREB phosphorylation and subsequent CREB-mediated transcriptions that may be beneficial for the AD synapses.

In the present study, we observed that both exercise and/or amyloid infusion did not produce any changes in the basal levels of ERK1/2 (phosphorylated and total) in both CA1 and DG areas, which resulted in similar p-ERK1/2 : t-ERK1/2 among all groups. However, others have reported ERK1/2 signaling dysfunction specific to AD in fibroblasts (Zhao et al., 2002) while voluntary running can markedly up-regulate the expression of ERK1/2 in exercised animals (Shen et al., 2001). This disagreement can be due to: 1) the time at which the protein level is measured as exercise-induced increase in levels of p-ERK1/2 is often delayed (Shen et al., 2001) and 2) Variations of technique employed in each study.

5.5.3.5. BDNF

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family, is a critical molecule for neuronal development and synaptic plasticity (Leal et al., 2013). Our AD model does not show a reduction in the basal levels of BDNF in both CA1 and DG areas indicating a possible early compensatory mechanism to oppose the effect of toxic amyloid peptides. However, in our study the exercised groups including exercise alone and Ex/Aβ rats showed a significant elevation in the basal levels of BDNF in both

hippocampal sub-regions compared to those of the sedentary controls. The pleiotropic effects of exercise-induced BDNF are well-documented, thus suggesting that upregulated BDNF might be a potential mechanism behind the neuroprotection of exercise in AD. It is not coincidental that the level of BDNF, a potent mediator of synaptic plasticity and memory, is highly elevated during exercise training (Castren et al., 1993b, Kesslak et al., 1998, Silhol et al., 2007). In addition to its central production and release, BDNF can be produced from peripheral non-neuronal tissues and cross the blood brain barrier via a "high capacity, saturable transport system" (Poduslo and Curran, 1996, Pan et al., 1998, Lommatzsch et al., 1999, Zoladz and Pilc, 2010). In brain tissues, BDNF exists in both monomeric and dimeric forms, which exert maximal effect on neuronal survival as tested in dorsal root ganglion neurons (Kolbeck et al., 1994). We observed that exercise altered the levels of monomers of BDNF in the same pattern of BDNF dimers in area CA1 suggesting a common function of both monomers and dimers at the pyramidal synapses. BDNF can act pre- or post-synaptically to modulate its own signaling or other pathways that are important in the learning and memory process including CaMKIV and CREB (Spencer et al., 2008, Williams et al., 2008, Cassilhas et al., 2012). In addition to its neurotrophic effect, BDNF also possesses metabotrophic properties. BDNF up-regulates the expression of energy-related molecules including AMP-activated protein kinase (AMPK), ubiquitous mitochondrial creatine kinase (uMtCK) and UCP2 (Gomez-Pinilla et al., 2008, Pedersen et al., 2009, Chaldakov, 2011). Thus, it is reasonable to suggest that depletion/disruption of BDNF expression/activity would significantly alter the action of these metabolic factors and eventually disrupt

learning and memory function. The present findings showed that the levels of BDNF after HFS were significantly elevated in the CA1 and DG areas of exercised rats compared to the sedentary groups. However, we observed that HFS failed to increase BDNF level in S-Control rats suggesting that the stimulation protocol is not strong enough to cause a significant change in BDNF protein levels. Moreover, HFS and exercise training seem to act in concert to increase the BDNF levels well beyond the normal levels in both normal-exercised and Aβ/exercised rats. This increase in BDNF may lead to improved performance in the RAWM and preservation of E-LTP in Ex/Aβ rats. Interestingly, 5 hrs after induction, the L-LTP protocol successfully produced a robust elevation in levels of BDNF in both CA1 and DG areas suggesting a prominent role of BDNF in long-lasting changes. In contrast, disruption of BDNF or its receptor (TrkB) impairs spatial memory and suppresses LTP expression (Zhou et al., 2000, Hennigan et al., 2009). The fact that these cognitive deficits can be restored by exogenous administration of BDNF suggests that BDNF plays an integral role in synaptic plasticity and memory (Shaw et al., 2003, Rex et al., 2006, Kline et al., 2010).

Moreover, the expression of BDNF mRNA significantly increases during physical activity suggesting its crucial role in mediating the neurotrophic effect of exercise. BDNF mRNA expression is directly proportional with training duration and inversely proportional with the detraining period (Widenfalk et al., 1999, Radak et al., 2006). Although both exercise paradigms are reported to enhance hippocampal BDNF levels (Khabour et al., 2010, Kim et al., 2010), voluntary exercise seems to increase BDNF expression to a higher extent compared to forced exercise (Leasure and Jones, 2008).

5.5.4. Is BDNF a potential mechanism for preventing the toxic effects of Aβ?

The level of BDNF is highly up-regulated in exercised animals which correlate with exercise-induced hippocampal neurogenesis in these animals. The pre- and post-synaptic actions of BDNF not only allow the enhanced LTP expression but also positively modulate intracellular molecular targets that are critical in maintain LTP expression, learning and memory (i.e. synapsin-1, CAMKII, CREB). In addition, acetylcholine- and mitochondrial proteins-mediated plasticity are also thought to involve action of BDNF (Massey et al., 2006, Vaynman et al., 2006a, Fernandes et al., 2008). Together, these lines of evidence signify a pivotal role of regular exercise in preventing Aβ toxicity via multiple cellular and molecular mechanisms that translate to the beneficial effect of exercise on cognitive and non-cognitive functions.

6. SUMMARY AND CONCLUSIONS

1. Our moderate exercise regimen per se did not produce a significant effect in cognitively normal rats. However, 4 weeks of treadmill exercise prevented cognitive and non-cognitive disturbances, basal synaptic transmission impairment, long-term potentiation (LTP) suppression and deleterious changes in LTP-related signaling pathways caused by i.c.v. infusion of $A\beta_{1-42}$. Hence, it seems that the exercise training protocol in this study is neuroprotective rather than neuroenhancing in the context of amyloid toxicity.
2. Our rat model of AD produces anxiety-like behaviors, which are totally prevented by regular exercise possibly by modulating cellular and molecular mechanisms of the synapses of pyramidal and granule cells. Interestingly, the model produces locomotor impairment but this deleterious effect is not removed by prior exercise suggesting our exercise regimen might not be strong enough to overcome and rescue motor neurons that have been chronically intoxicated with $A\beta_{1-42}$.
3. At the molecular level, by decreasing the basal levels of APP and BACE-1 in CA1 and DG neurons, exercise drives the processing of APP more toward the non-amyloidogenic pathway, which contributes to the beneficial effect of exercise in AD pathology.
4. The reduction in the basal levels of p-CaMKII in $A\beta$ rats suggests a functional disruption of AD pathology, which probably leads to impaired E-LTP in both CA1

and DG areas. Regular exercise counteracts this deleterious effect by restoring the basal level of p-CaMKII in Aβ rats back to normal.

5. The aberrant increase in basal levels of calcineurin in Aβ rats correlates with impaired phosphorylation of CaMKII upon amyloid infusion. This negative regulation of calcineurin is not seen in Ex/Aβ rats may indicate that by re-establishing the kinase-phosphatase balance, regular exercise prevents E-LTP inhibition, which probably is due to limited p-CaMKII and increased calcineurin availability.
6. Our electrophysiological findings show suppressed E-LTP in both CA1 and DG areas but pyramidal neurons of area CA1 seem to suffer the damage to higher extent compared to the granule cells of DG area. Perhaps, physiological and exercise-induced neurogenesis in the DG explains partly this regional effect of exercise. The E-LTP suppression caused by $A\beta_{1-42}$ infusion maybe due to inability of HFS to appreciably increase the level of p-CaMKII in Aβ rats. In contrast, S-Ex/Aβ rats displayed a normal protein expression of p-CaMKII after E-LTP induction similar to those of control and exercise rats, possibly by preventing the abnormal HFS-induced increase in levels of calcineurin.
7. Continuous amyloid infusion negatively regulates the basal protein expression of p-CREB in both CA1 and DG areas, which is attributed to an impaired signaling of CREB upstream modulator such as CaMKIV. 4 weeks of treadmill exercise prevents this CREB/CaMKIV signaling impairment by normalizing the basal levels

of both p-CREB and CaMKIV in CA1 and DG areas in Ex/Aβ rats, which in turn results in intact spatial long-term memory in these animals.

8. MHFS induces a robust elevation in levels of p-CREB and CaMKIV in all groups except the Aβ group suggesting that by positively regulating the levels of these key signaling molecules, exercise training produces normal L-LTP in Aβ rats. Additionally, MHFS-induced increase in levels of CaMKIV in S-Ex/Aβ rats also explains the preservation of p-CREB levels and thus normal L-LTP in these animals.
9. Altogether, these behavioral, electrophysiological, and molecular findings consistently demonstrate the neuroprotective role of regular exercise in AD-like pathology, which could be very useful for possible development of exercise mimetic strategies for the disease itself and other learning and memory deficit pathologies.

	Primary antibody	Secondary antibody
P-CaMKII	Mouse monoclonal antibody anti-P-CaMKII (1:500; Santa Cruz Biotechnology, Inc. CA)	Anti-mouse antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
T- CaMKII	Rabbit polyclonal antibody anti-CaMKII (1:1000; Santa Cruz Biotechnology, Inc. CA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
Calcineurin	Rabbit polyclonal anti-calcineurin antibody (1:1000; Upstate Biotechnology, NY)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
BDNF	Rabbit polyclonal anti-BDNF (1:500; Santa Cruz Biotechnology, Inc. CA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
P-CREB	Rabbit polyclonal antibody anti-P-CREB (1:1000; Milipore, MA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
T- CREB	Rabbit polyclonal antibody anti-CREB (1:100; Santa Cruz Biotechnology, Inc. CA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
CaMKIV	Rabbit polyclonal anti-CaMKIV (1:1000; Cell Signaling Technology, MA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
P/T- ERK1/2	Rabbit monoclonal anti-P- or T-ERK1/2 (1:1000; Cell Signaling Technology, MA)	Anti-rabbit antibodyHRP (1:5000; Santa Cruz Biotechnology, Inc. CA)
GAPDH	Rabbit monoclonal antibody against GAPDH (1:2000; Millipore, MA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
APP	Rabbit monoclonal antibody against APP (1:1000; Abcam, MA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)
BACE-1	Rabbit monoclonal antibody against APP (1:500; Abcam, MA)	Anti-rabbit antibody (1:5000; Santa Cruz Biotechnology, Inc. CA)

Table 1. Antibodies, dilutions, and sources. Summary of primary and secondary antibodies used in detecting the levels of the given molecules.

Group	E-LTP expression in CA1	E-LTP expression in DG	L-LTP expression in CA1	L-LTP expression in DG
Control	fEPSP-141 pSpike-185	fEPSP-142 pSpike-226	fEPSP-156 pSpike-210	fEPSP-138 pSpike-217
Aβ	fEPSP-107 pSpike-93	fEPSP-100 pSpike-255	fEPSP-109 pSpike-91	fEPSP-97 pSpike-225
Exercise	fEPSP-139 pSpike-169	fEPSP-136 pSpike-231	fEPSP-140 pSpike-230	fEPSP-138 pSpike-219
Ex/ Aβ	fEPSP-140 pSpike- 133	fEPSP-138 pSpike-233	fEPSP-146 pSpike-139	fEPSP-157 pSpike-196

Table 2. The effects of amyloid infusion and/or exercise training on E-LTP and L-LTP expression in the CA1 and DG areas. The numerical value is compared to baseline (100%). fEPSP slope and pspike amplitude represent synaptic strength and the number of neurons that reach threshold and fire respectively. Red indicates significant difference from all groups.

	Aβ		Exercise		Exercise/ Aβ	
Area	CA1	DG	CA1	DG	CA1	DG
p-CaMKII	↓	↓	No change	No change	No change	No change
t-CaMKII	No change	No change	No change	No change	No change	No change
p-CaMKII: t-CaMKII	↓	↓	No change	No change	No change	No change
Calcineurin	↑	↑	No change	No change	No change	No change
BDNF	No change	No change	↑	↑	↑	↑
p-CREB	↓	↓	No change	No change	No change	No change
t-CREB	No change	No change	No change	No change	No change	No change
p-CREB:t-CREB	↓	↓	No change	No change	No change	No change
CaMKIV	↓	↓	No change	No change	No change	No change
p-ERK1/2	No change	No change	No change	No change	No change	No change
t-ERK1/2	No change	No change	No change	No change	No change	No change
p-ERK1/2:t-ERK1/2	No change	No change	No change	No change	No change	No change
APP	↑	↑	No change	No change	No change	No change
BACE-1	↑	↑	No change	No change	No change	No change

Table 3. The effects of amyloid infusion and/or exercise training on the basal levels of signaling molecules in the CA1 and DG areas compared to control rats.

	S-Control		S-Aβ		S-Exercise		S-Exercise/ Aβ	
Area	CA1	DG	CA1	DG	CA1	DG	CA1	DG
p-CaMKII	+	+	No change	No change	+	+	+	+
t-CaMKII	+	+	+	+	+	+	+	+
Calcineurin	+	+	+	+	No change	No change	No change	No change
BDNF	No change	No change	No change	No change	+	+	+	+

Table 4. The effects of amyloid infusion and/or exercise training on the levels of signaling molecules in the CA1 and DG areas after E-LTP induction via single HFS compared to unstimulated control rats. (+) indicates increased expression compared to unstimulated controls.

	S-Control		S-Aβ		S-Exercise		S-Exercise/ Aβ	
Area	CA1	DG	CA1	DG	CA1	DG	CA1	DG
p-CREB	+	+	No change	No change	+	+	+	+
t-CREB	+	+	+	+	+	+	+	+
CaMKIV	+	+	No change	No change	+	+	+	+
BDNF	+	+	No change	No change	+	+	+	+

Table 5. The effects of amyloid infusion and/or exercise training on the levels of signaling molecules in the CA1 and DG areas after L-LTP induction via MHFS compared to unstimulated control rats. (+) indicates increased expression compared to unstimulated controls.

7. REFERENCE

Abeti R, Abramov AY, Duchen MR (2011) Beta-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. Brain 134:1658-1672.

Adlard PA, Parncutt JM, Finkelstein DI, Bush AI (2010) Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? The Journal of Neuroscience 30:1631-1636.

Adlard PA, Perreau VM, Pop V, Cotman CW (2005) Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. The Journal of Neuroscience 25:4217-4221.

Aguiar AS, Jr., Araujo AL, da-Cunha TR, Speck AE, Ignacio ZM, De-Mello N, Prediger RD (2009) Physical exercise improves motor and short-term social memory deficits in reserpinized rats. Brain Research Bulletin 79:452-457.

Al-Jarrah M, Jamous M, Al Zailaey K, Bweir SO (2010) Endurance exercise training promotes angiogenesis in the brain of chronic/progressive mouse model of Parkinson's Disease. NeuroRehabilitation 26:369-373.

Alaei H, Borjeian L, Azizi M, Orian S, Pourshanazari A, Hanninen O (2006) Treadmill running reverses retention deficit induced by morphine. European Journal of Pharmacology 536:138-141.

Alamed J, Wilcock DM, Diamond DM, Gordon MN, Morgan D (2006) Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice. Nature Protocols 1:1671-1679.

Albeck DS, Sano K, Prewitt GE, Dalton L (2006) Mild forced treadmill exercise enhances spatial learning in the aged rat. Behavioral Brain Research 168:345-348.

Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiological Reviews 89:121-145.

Aleisa AM, Alzoubi KH, Alkadhi KA (2006) Chronic but not acute nicotine treatment reverses stress-induced impairment of LTP in anesthetized rats. Brain Research 1097:78-84.

Aliev G, Obrenovich ME, Smith MA, Perry G (2003) Hypoperfusion, Mitochondria Failure, Oxidative Stress, and Alzheimer Disease. Journal of Biomedicine & Biotechnology 2003:162-163.

Alkadhi K, Alzoubi KH, Srivareerat M, Tran TT (2011) Chronic psychosocial stress exacerbates impairment of synaptic plasticity in beta-amyloid rat model of Alzheimer's disease: prevention by nicotine. Current Alzheimer Research 8:718-731.

Alkadhi KA, Srivareerat M, Tran TT (2010) Intensification of long-term memory deficit by chronic stress and prevention by nicotine in a rat model of Alzheimer's disease. Molecular and Cellular Neurosciences 45:289-296.

Allen SJ, MacGowan SH, Tyler S, Wilcock GK, Robertson AG, Holden PH, Smith SK, Dawbarn D (1997) Reduced cholinergic function in normal and Alzheimer's disease brain is associated with apolipoprotein E4 genotype. Neuroscience Letters 239:33-36.

Allinson TM, Parkin ET, Turner AJ, Hooper NM (2003) ADAMs family members as amyloid precursor protein alpha-secretases. Journal of Neuroscience Research 74:342-352.

Alzoubi KH, Abdul-Razzak KK, Khabour OF, Al-Tuweiq GM, Alzubi MA, Alkadhi KA (2013) Caffeine prevents cognitive impairment induced by chronic psychosocial stress and/or high fat-high carbohydrate diet. Behavioural Brain Research 237:7-14.

Alzoubi KH, Aleisa AM, Alkadhi KA (2005) Impairment of long-term potentiation in the CA1, but not dentate gyrus, of the hippocampus in Obese Zucker rats: role of calcineurin and phosphorylated CaMKII. Journal of Molecular Neuroscience : MN 27:337-346.

Alzoubi KH, Aleisa AM, Alkadhi KA (2007) Nicotine prevents disruption of the late phase LTP-related molecular cascade in adult-onset hypothyroidism. Hippocampus 17:654-664.

Alzoubi KH, Aleisa AM, Alkadhi KA (2010) In vivo expression of ganglionic long-term potentiation in superior cervical ganglia from hypertensive aged rats. Neurobiology of Aging 31:805-812.

Alzoubi KH, Alhaider IA, Tran TT, Mosely A, Alkadhi KK (2011) Impaired neural transmission and synaptic plasticity in superior cervical ganglia from beta-amyloid rat model of Alzheimer's disease. Current Alzheimer Research 8:377-384.

Alzoubi KH, Alkadhi KA (2007) A critical role of CREB in the impairment of late-phase LTP by adult onset hypothyroidism. Experimental Neurology203:63-71.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31:571-591.

Araujo J, Caio Maximino, Thiago Marques de Brito, Annanda Waneza Batista da Silva, Karen Renata Matos Oliveira, Evander de Jesus Oliveira Batista, Silvio Morato, Anderson Manoel Herculano, Jr. AG (2012) Behavioral and Pharmacological Aspects of Anxiety in the Light/Dark Preference Test. Neuromethods 66:191-202.

Ardiles AO, Tapia-Rojas CC, Mandal M, Alexandre F, Kirkwood A, Inestrosa NC, Palacios AG (2012) Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 109:13835-13840.

Arrazola MS, Varela-Nallar L, Colombres M, Toledo EM, Cruzat F, Pavez L, Assar R, Aravena A, Gonzalez M, Montecino M, Maass A, Martinez S, Inestrosa NC (2009) Calcium/calmodulin-dependent protein kinase type IV is a target gene of the Wnt/beta-catenin signaling pathway. Journal of Cellular Physiology 221:658-667.

Asmundson GJ, Fetzner MG, Deboer LB, Powers MB, Otto MW, Smits JA (2013) Let'S get physical: a contemporary review of the anxiolytic effects of exercise for anxiety and its disorders. Depression and anxiety 30:362-373.

Auerbach JM, Segal M (1996) Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. The Journal of Physiology 492 (Pt 2):479-493.

Auffret A, Gautheron V, Mattson MP, Mariani J, Rovira C (2010) Progressive age-related impairment of the late long-term potentiation in Alzheimer's disease presenilin-1 mutant knock-in mice. Journal of Alzheimer's Disease 19:1021-1033.

Bach ME, Hawkins RD, Osman M, Kandel ER, Mayford M (1995) Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. Cell 81:905-915.

Bailey CH, Bartsch D, Kandel ER (1996) Toward a molecular definition of long-term memory storage. Proceedings of the National Academy of Sciences of the United States of America 93:13445-13452.

Bangsbo J, Krustrup P, Gonzalez-Alonso J, Saltin B (2001) ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. American Journal of Physiology Endocrinology and Metabolism 280:E956-964.

Barco A, Patterson SL, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER (2005) Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48:123-137.

Bartlett JD, Louhelainen J, Iqbal Z, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP (2013) Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. American Journal of Physiology Regulatory, Integrative and Comparative Physiology 304:R450-458.

Bartley CA, Hay M, Bloch MH (2013) Meta-analysis: Aerobic exercise for the treatment of anxiety disorders. Progress in Neuro-psychopharmacology & Biological Psychiatry 45C:34-39.

Bast T (2007) Toward an integrative perspective on hippocampal function: from the rapid encoding of experience to adaptive behavior. Reviews in The Neurosciences 18:253-281.

Bechara RG, Kelly AM (2013) Exercise improves object recognition memory and induces BDNF expression and cell proliferation in cognitively enriched rats. Behavioural Brain Research 245:96-100.

Belmeguenai A, Hansel C (2005) A role for protein phosphatases 1, 2A, and 2B in cerebellar long-term potentiation. The Journal of Neuroscience 25:10768-10772.

Bengtson CP, Bading H (2012) Nuclear calcium signaling. Advances in Experimental Medicine and Biology 970:377-405.

Berberich S, Jensen V, Hvalby O, Seeburg PH, Kohr G (2007) The role of NMDAR subtypes and charge transfer during hippocampal LTP induction. Neuropharmacology 52:77-86.

Berchtold NC, Castello N, Cotman CW (2010) Exercise and time-dependent benefits to learning and memory. Neuroscience 167:588-597.

Berkin KE, Walker G, Inglis GC, Ball SG, Thomson NC (1988) Circulating adrenaline and noradrenaline concentrations during exercise in patients with exercise induced asthma and normal subjects. Thorax 43:295-299.

Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT (1990) Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. Proceedings of the National Academy of Sciences of the United States of America 87:5568-5572.

Blackshear AL, Xu W, Anderson M, Xu F, Previti ML, Van Nostrand WE, Robinson JK (2011) A novel operant testing regimen for multi-construct cognitive characterization of a murine model of Alzheimer's amyloid-related behavioral impairment. Neurobiology of Learning and Memory 96:443-451.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.

Bobinski M, de Leon MJ, Wegiel J, Desanti S, Convit A, Saint Louis LA, Rusinek H, Wisniewski HM (2000) The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. Neuroscience 95:721-725.

Bodendorf U, Danner S, Fischer F, Stefani M, Sturchler-Pierrat C, Wiederhold KH, Staufenbiel M, Paganetti P (2002) Expression of human beta-secretase in the mouse brain increases the steady-state level of beta-amyloid. Journal of Neurochemistry 80:799-806.

Borchelt DR, Ratovitski T, van Lare J, Lee MK, Gonzales V, Jenkins NA, Copeland NG, Price DL, Sisodia SS (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 19:939-945.

Bortolotto ZA, Nistico R, More JC, Jane DE, Collingridge GL (2005) Kainate receptors and mossy fiber LTP. Neurotoxicology 26:769-777.

Bowen DM, Sims NR, Davison AN (1982) Neurochemistry of Alzheimer's disease: an update. Experimental Brain Research 5:127-132.

Brady AM, Saul RD, Wiest MK (2010) Selective deficits in spatial working memory in the neonatal ventral hippocampal lesion rat model of schizophrenia. Neuropharmacology 59:605-611.

Broman-Fulks JJ, Berman ME, Rabian BA, Webster MJ (2004) Effects of aerobic exercise on anxiety sensitivity. Behaviour Research and Therapy 42:125-136.

Broman-Fulks JJ, Storey KM (2008) Evaluation of a brief aerobic exercise intervention for high anxiety sensitivity. Anxiety, Stress, and Coping 21:117-128.

Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer's disease. Alzheimer’s & Dementia 3:186-191.

Brouillette J, Caillierez R, Zommer N, Alves-Pires C, Benilova I, Blum D, De Strooper B, Buee L (2012) Neurotoxicity and memory deficits induced by soluble low-molecular-weight amyloid-beta1-42 oligomers are revealed in vivo by using a novel animal model. The Journal of Neuroscience 32:7852-7861.

Buggia-Prevot V, Sevalle J, Rossner S, Checler F (2008) NFkappaB-dependent control of BACE1 promoter transactivation by Abeta42. The Journal of Biological Chemistry 283:10037-10047.

Bullitt E, Rahman FN, Smith JK, Kim E, Zeng D, Katz LM, Marks BL (2009) The effect of exercise on the cerebral vasculature of healthy aged subjects as visualized by MR angiography. American Journal of Neuroradiology 30:1857-1863.

Buresova O, Bures J, Oitzl MS, Zahalka A (1985) Radial maze in the water tank: an aversively motivated spatial working memory task. Physiology & Behavior 34:1003-1005.

Burns A, Jacoby R, Levy R (1990) Psychiatric phenomena in Alzheimer's disease. I: Disorders of thought content. The British Journal of Psychiatry. 157:72-76, 92-74.

Cakir B, Kasimay O, Kolgazi M, Ersoy Y, Ercan F, Yegen BC (2010) Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise. Cell biochemistry and function 28:469-479.

Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH (2011) Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic

degeneration in a mouse model of Alzheimer's disease. Human Molecular Genetics 20:4515-4529.

Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N, Jr., Frautschy SA, Cole GM (2005) Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. European Journal of Neuroscience 22:617-626.

Cammarota M, Bevilaqua LR, Viola H, Kerr DS, Reichmann B, Teixeira V, Bulla M, Izquierdo I, Medina JH (2002) Participation of CaMKII in neuronal plasticity and memory formation. Cellular and Molecular Neurobiology 22:259-267.

Campbell-O'Sullivan SP, Constantin-Teodosiu D, Peirce N, Greenhaff PL (2002) Low intensity exercise in humans accelerates mitochondrial ATP production and pulmonary oxygen kinetics during subsequent more intense exercise. The Journal of Physiology 538:931-939.

Canudas AM, Gutierrez-Cuesta J, Rodriguez MI, Acuna-Castroviejo D, Sureda FX, Camins A, Pallas M (2005) Hyperphosphorylation of microtubule-associated protein tau in senescence-accelerated mouse (SAM). Mechanisms of Ageing and Development 126:1300-1304.

Capone R JH, Kotler SA, Connelly L, Teran Arce F, Ramachandran S, Kagan BL, Nussinov R, Lal R (2012a) All-d-Enantiomer of β-Amyloid Peptide Forms Ion Channels in Lipid Bilayers. Journal of Chemical Theory and Computation 8:1143-1152.

Capone R JH, Kotler SA, Kagan BL, Nussinov R, Lal R (2012b) Probing structural features of Alzheimer's amyloid-β pores in bilayers using site-specific amino acid substitutions. Biochemistry 51:776-785.

Capsoni S, Tiveron C, Vignone D, Amato G, Cattaneo A (2010) Dissecting the involvement of tropomyosin-related kinase A and p75 neurotrophin receptor signaling in NGF deficit-induced neurodegeneration. Proceedings of the National Academy of Sciences of the United States of America 107:12299-12304.

Cassilhas RC, Lee KS, Fernandes J, Oliveira MG, Tufik S, Meeusen R, de Mello MT (2012) Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. Neuroscience 202:309-317.

Castren E, da Penha Berzaghi M, Lindholm D, Thoenen H (1993a) Differential effects of MK-801 on brain-derived neurotrophic factor mRNA levels in different regions of the rat brain. Experimental Neurology 122:244-252.

Castren E, Pitkanen M, Sirvio J, Parsadanian A, Lindholm D, Thoenen H, Riekkinen PJ (1993b) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport 4:895-898.

Cavallucci V, D'Amelio M, Cecconi F (2012) Abeta toxicity in Alzheimer's disease. Molecular Neurobiology 45:366-378.

Cavedo E, Boccardi M, Ganzola R, Canu E, Beltramello A, Caltagirone C, Thompson PM, Frisoni GB (2011) Local amygdala structural differences with 3T MRI in patients with Alzheimer disease. Neurology 76:727-733.

Cechetti F, Worm PV, Elsner VR, Bertoldi K, Sanches E, Ben J, Siqueira IR, Netto CA (2012) Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat. Neurobiology of Learning and Memory 97:90-96.

Cetinkaya C, Sisman AR, Kiray M, Camsari UM, Gencoglu C, Baykara B, Aksu I, Uysal N (2013) Positive effects of aerobic exercise on learning and memory functioning, which correlate with hippocampal IGF-1 increase in adolescent rats. Neuroscience Letters.

Chae CH, Jung SL, An SH, Park BY, Wang SW, Cho IH, Cho JY, Kim HT (2009) Treadmill exercise improves cognitive function and facilitates nerve growth factor signaling by activating mitogen-activated protein kinase/extracellular signal-regulated kinase1/2 in the streptozotocin-induced diabetic rat hippocampus. Neuroscience 164:1665-1673.

Chae CH, Kim HT (2009) Forced, moderate-intensity treadmill exercise suppresses apoptosis by increasing the level of NGF and stimulating phosphatidylinositol 3-kinase signaling in the hippocampus of induced aging rats. Neurochemistry International 55:208-213.

Chaldakov G (2011) The metabotrophic NGF and BDNF: an emerging concept. Archives Italiennes de Biologie 149:257-263.

Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TV, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nature Neuroscience2:271-276.

Chen MJ, Russo-Neustadt AA (2009) Running exercise-induced up-regulation of hippocampal brain-derived neurotrophic factor is CREB-dependent. Hippocampus 19:962-972.

Chen QS, Wei WZ, Shimahara T, Xie CW (2002) Alzheimer amyloid beta-peptide inhibits the late phase of long-term potentiation through calcineurin-dependent mechanisms in the hippocampal dentate gyrus. Neurobiol Learn Mem 77:354-371.

Chen Y, Liang Z, Blanchard J, Dai CL, Sun S, Lee MH, Grundke-Iqbal I, Iqbal K, Liu F, Gong CX (2013) A non-transgenic mouse model (icv-STZ mouse) of Alzheimer's disease: similarities to and differences from the transgenic model (3xTg-AD mouse). Molecular neurobiology 47:711-725.

Cheng ST, Chow PK, Song YQ, Yu EC, Chan AC, Lee TM, Lam JH (2012a) Mental and Physical Activities Delay Cognitive Decline in Older Persons With Dementia. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry.

Cheng ST, Kwok T, Lam LC (2012b) Neuropsychiatric symptom clusters of Alzheimer's disease in Hong Kong Chinese: prevalence and confirmatory factor analysis of the Neuropsychiatric Inventory. International psychogeriatrics / IPA 24:1465-1473.

Chin ER (2004) The role of calcium and calcium/calmodulin-dependent kinases in skeletal muscle plasticity and mitochondrial biogenesis. The Proceedings of the Nutrition Society 63:279-286.

Chirita CN, Necula M, Kuret J (2003) Anionic micelles and vesicles induce tau fibrillization in vitro. The Journal of biological chemistry 278:25644-25650.

Chong SA, Benilova I, Shaban H, De Strooper B, Devijver H, Moechars D, Eberle W, Bartic C, Van Leuven F, Callewaert G (2011) Synaptic dysfunction in hippocampus of transgenic mouse models of Alzheimer's disease: a multi-electrode array study. Neurobiology of disease 44:284-291.

Chow FA, Anderson KA, Noeldner PK, Means AR (2005) The autonomous activity of calcium/calmodulin-dependent protein kinase IV is required for its role in transcription. The Journal of biological chemistry 280:20530-20538.

Christie BR, Eadie BD, Kannangara TS, Robillard JM, Shin J, Titterness AK (2008) Exercising our brains: how physical activity impacts synaptic plasticity in the dentate gyrus. Neuromolecular medicine 10:47-58.

Chung JY, Kim MW, Bang MS, Kim M (2010) The effect of exercise on trkA in the contralateral hemisphere of the ischemic rat brain. Brain Research 1353:187-193.

Cirrito JR, May PC, O'Dell MA, Taylor JW, Parsadanian M, Cramer JW, Audia JE, Nissen JS, Bales KR, Paul SM, DeMattos RB, Holtzman DM (2003) In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:8844-8853.

Clark PJ, Brzezinska WJ, Puchalski EK, Krone DA, Rhodes JS (2009) Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. Hippocampus 19:937-950.

Coelho FG, Gobbi S, Andreatto CA, Corazza DI, Pedroso RV, Santos-Galduroz RF (2013) Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): a systematic review of experimental studies in the elderly. Archives of gerontology and geriatrics 56:10-15.

Colgin LL, Kubota D, Lynch G (2003) Cholinergic plasticity in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 100:2872-2877.

Connelly L, Jang H, Arce FT, Capone R, Kotler SA, Ramachandran S, Kagan BL, Nussinov R, Lal R (2012) Atomic force microscopy and MD simulations reveal pore-like structures of all-D-enantiomer of Alzheimer's beta-amyloid peptide: relevance to the ion channel mechanism of AD pathology. J Phys Chem B 116:1728-1735.

Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J, Stephan DA (2007) A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. The Journal of clinical psychiatry 68:613-618.

Couchonnal LF, Anderson ME (2008) The role of calmodulin kinase II in myocardial physiology and disease. Physiology 23:151-159.

Coulson DT, Beyer N, Quinn JG, Brockbank S, Hellemans J, Irvine GB, Ravid R, Johnston JA (2010) BACE1 mRNA expression in Alzheimer's disease postmortem brain tissue. Journal of Alzheimer's disease : JAD 22:1111-1122.

Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, Morris JC (2006) Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. The American journal of psychiatry 163:2164-2169.

Cui Z, Wang H, Tan Y, Zaia KA, Zhang S, Tsien JZ (2004) Inducible and reversible NR1 knockout reveals crucial role of the NMDA receptor in preserving remote memories in the brain. Neuron 41:781-793.

Curlik DM, 2nd, Shors TJ (2013) Training your brain: Do mental and physical (MAP) training enhance cognition through the process of neurogenesis in the hippocampus? Neuropharmacology 64:506-514.

Czurko A, Hirase H, Csicsvari J, Buzsaki G (1999) Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. European Journal of Neuroscience 11:344-352.

Daftary SS, Gore AC (2005) IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Experimental biology and medicine 230:292-306.

Dao AT, Zagaar MA, Levine AT, Salim S, Eriksen JL, Alkadhi KA (2013) Treadmill exercise prevents learning and memory impairment in Alzheimer's disease-like pathology. Current Alzheimer research 10:507-515.

Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathe AA, Johns CA, Horvath TB (1986) Cortisol and Alzheimer's disease, I: Basal studies. The American journal of psychiatry 143:300-305.

Davis S, Butcher SP, Morris RG (1992) The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience 12:21-34.

Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK (2006) Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:9057-9068.

Dewachter I, Filipkowski RK, Priller C, Ris L, Neyton J, Croes S, Terwel D, Gysemans M, Devijver H, Borghgraef P, Godaux E, Kaczmarek L, Herms J, Van Leuven F (2009) Deregulation of NMDA-receptor function and down-stream signaling in APP[V717I] transgenic mice. Neurobiol Aging 30:241-256.

Diamond DM, Park CR, Heman KL, Rose GM (1999) Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus 9:542-552.

Dietrich A, McDaniel WF (2004) Endocannabinoids and exercise. British journal of sports medicine 38:536-541.

Dietrich MO, Andrews ZB, Horvath TL (2008) Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:10766-10771.

Dietrich WF, Miller J, Steen R, Merchant MA, Damron-Boles D, Husain Z, Dredge R, Daly MJ, Ingalls KA, O'Connor TJ (1996) A comprehensive genetic map of the mouse genome. Nature 380:149-152.

Dimatelis JJ, Hendricks S, Hsieh J, Vlok NM, Bugarith K, Daniels WM, Russell VA (2013) Exercise partly reverses the effect of maternal separation on hippocampal proteins in 6-hydroxydopamine-lesioned rat brain. Experimental physiology 98:233-244.

Ding Q, Vaynman S, Akhavan M, Ying Z, Gomez-Pinilla F (2006a) Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience 140:823-833.

Ding YH, Li J, Zhou Y, Rafols JA, Clark JC, Ding Y (2006b) Cerebral angiogenesis and expression of angiogenic factors in aging rats after exercise. Current neurovascular research 3:15-23.

Doherty GH, Beccano-Kelly D, Yan SD, Gunn-Moore FJ, Harvey J (2013) Leptin prevents hippocampal synaptic disruption and neuronal cell death induced by amyloid beta. Neurobiology of aging 34:226-237.

Dominguez-del-Toro E, Rodriguez-Moreno A, Porras-Garcia E, Sanchez-Campusano R, Blanchard V, Laville M, Bohme GA, Benavides J, Delgado-Garcia JM (2004) An in vitro and in vivo study of early deficits in associative learning in transgenic mice that over-express a mutant form of human APP associated with Alzheimer's disease. The European journal of neuroscience 20:1945-1952.

Dominguez D, Tournoy J, Hartmann D, Huth T, Cryns K, Deforce S, Serneels L, Camacho IE, Marjaux E, Craessaerts K, Roebroek AJ, Schwake M, D'Hooge R, Bach P, Kalinke U, Moechars D, Alzheimer C, Reiss K, Saftig P, De Strooper B (2005) Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice. The Journal of biological chemistry 280:30797-30806.

Drachman DA, Leavitt J (1974) Human memory and the cholinergic system. A relationship to aging? Archives of neurology 30:113-121.

Droste SK, Collins A, Lightman SL, Linthorst AC, Reul JM (2009) Distinct, time-dependent effects of voluntary exercise on circadian and ultradian rhythms and stress responses of free corticosterone in the rat hippocampus. Endocrinology 150:4170-4179.

Drumond LE, Mourao FA, Leite HR, Abreu RV, Reis HJ, Moraes MF, Pereira GS, Massensini AR (2012) Differential effects of swimming training on neuronal calcium sensor-1 expression in rat hippocampus/cortex and in object recognition memory tasks. Brain Research bulletin 88:385-391.

Du K, Asahara H, Jhala US, Wagner BL, Montminy M (2000) Characterization of a CREB gain-of-function mutant with constitutive transcriptional activity in vivo. Molecular and cellular biology 20:4320-4327.

Durany N, Michel T, Kurt J, Cruz-Sanchez FF, Cervas-Navarro J, Riederer P (2000) Brain-derived neurotrophic factor and neurotrophin-3 levels in Alzheimer's disease brains. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 18:807-813.

Durkin JT, Murthy S, Husten EJ, Trusko SP, Savage MJ, Rotella DP, Greenberg BD, Siman R (1999) Rank-order of potencies for inhibition of the secretion of abeta40 and abeta42 suggests that both are generated by a single gamma-secretase. The Journal of biological chemistry 274:20499-20504.

Eadie BD, Redila VA, Christie BR (2005) Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. The Journal of comparative neurology 486:39-47.

Echeverria V, Cuello AC (2002) Intracellular A-beta amyloid, a sign for worse things to come? Molecular neurobiology 26:299-316.

Egan B, Carson BP, Garcia-Roves PM, Chibalin AV, Sarsfield FM, Barron N, McCaffrey N, Moyna NM, Zierath JR, O'Gorman DJ (2010) Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. The Journal of physiology 588:1779-1790.

Ekstrand J, Hellsten J, Tingstrom A (2008) Environmental enrichment, exercise and corticosterone affect endothelial cell proliferation in adult rat hippocampus and prefrontal cortex. Neuroscience letters 442:203-207.

Elvander-Tottie E, Eriksson TM, Sandin J, Ogren SO (2006) N-methyl-D-aspartate receptors in the medial septal area have a role in spatial and emotional learning in the rat. Neuroscience 142:963-978.

English JD, Sweatt JD (1997) A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. The Journal of biological chemistry 272:19103-19106.

Erickson KI, Weinstein AM, Lopez OL (2012) Physical activity, brain plasticity, and Alzheimer's disease. Archives of medical research 43:615-621.

Ewers M, Cheng X, Zhong Z, Nural HF, Walsh C, Meindl T, Teipel SJ, Buerger K, He P, Shen Y, Hampel H (2011) Increased CSF-BACE1 activity associated with decreased hippocampus volume in Alzheimer's disease. Journal of Alzheimer's disease : JAD 25:373-381.

Eyre MD, Richter-Levin G, Avital A, Stewart MG (2003) Morphological changes in hippocampal dentate gyrus synapses following spatial learning in rats are transient. The European journal of neuroscience 17:1973-1980.

Falls WA, Fox JH, MacAulay CM (2010) Voluntary exercise improves both learning and consolidation of cued conditioned fear in C57 mice. Behavioural Brain Research 207:321-331.

Fan N, Yang H, Zhang J, Chen C (2010) Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Delta9-THC exposure-impaired hippocampal synaptic plasticity. Journal of neurochemistry 112:691-702.

Faraji J, Lehmann H, Metz GA, Sutherland RJ (2008) Rats with hippocampal lesion show impaired learning and memory in the ziggurat task: a new task to evaluate spatial behavior. Behavioural Brain Research 189:17-31.

Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR (2004) Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. Neuroscience 124:71-79.

Fernandes CC, Pinto-Duarte A, Ribeiro JA, Sebastiao AM (2008) Postsynaptic action of brain-derived neurotrophic factor attenuates alpha7 nicotinic acetylcholine receptor-mediated responses in hippocampal interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:5611-5618.

Filali M, Lalonde R, Theriault P, Julien C, Calon F, Planel E (2012) Cognitive and non-cognitive behaviors in the triple transgenic mouse model of Alzheimer's disease expressing mutated APP, PS1, and Mapt (3xTg-AD). Behavioural Brain Research 234:334-342.

Filley CM, Rollins YD, Anderson CA, Arciniegas DB, Howard KL, Murrell JR, Boyer PJ, Kleinschmidt-DeMasters BK, Ghetti B (2007) The genetics of very early onset Alzheimer disease. Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology 20:149-156.

Fimia GM, Morlon A, Macho B, De Cesare D, Sassone-Corsi P (2001) Transcriptional cascades during spermatogenesis: pivotal role of CREM and ACT. Molecular and cellular endocrinology 179:17-23.

Fukushima H, Maeda R, Suzuki R, Suzuki A, Nomoto M, Toyoda H, Wu LJ, Xu H, Zhao MG, Ueda K, Kitamoto A, Mamiya N, Yoshida T, Homma S, Masushige S, Zhuo M, Kida S (2008) Upregulation of calcium/calmodulin-dependent protein kinase IV improves memory formation and rescues memory loss with aging. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:9910-9919.

Games D, Khan KM, Soriano FG, Keim PS, Davis DL, Bryant K, Lieberburg I (1992) Lack of Alzheimer pathology after beta-amyloid protein injections in rat brain. Neurobiology of aging 13:569-576.

Garcia-Escudero V, Martin-Maestro P, Perry G, Avila J (2013) Deconstructing mitochondrial dysfunction in Alzheimer disease. Oxidative medicine and cellular longevity 2013:162152.

Garcia-Mesa Y, Gimenez-Llort L, Lopez LC, Venegas C, Cristofol R, Escames G, Acuna-Castroviejo D, Sanfeliu C (2012) Melatonin plus physical exercise are highly neuroprotective in the 3xTg-AD mouse. Neurobiology of aging 33:1124 e1113-1129.

Garre-Olmo J, Lopez-Pousa S, Vilalta-Franch J, de Gracia Blanco M, Vilarrasa AB (2010) Grouping and trajectories of the neuropsychiatric symptoms in patients with Alzheimer's disease, part I: symptom clusters. Journal of Alzheimer's disease : JAD 22:1157-1167.

Ge S, Sailor KA, Ming GL, Song H (2008) Synaptic integration and plasticity of new neurons in the adult hippocampus. The Journal of physiology 586:3759-3765.

Gendron TF, Petrucelli L (2009) The role of tau in neurodegeneration. Molecular neurodegeneration 4:13.

Gerges NZ, Alkadhi KA (2004) Hypothyroidism impairs late LTP in CA1 region but not in dentate gyrus of the intact rat hippocampus: MAPK involvement. Hippocampus 14:40-45.

Gerges NZ, Alzoubi KH, Alkadhi KA (2005) Role of phosphorylated CaMKII and calcineurin in the differential effect of hypothyroidism on LTP of CA1 and dentate gyrus. Hippocampus 15:480-490.

Gerges NZ, Alzoubi KH, Park CR, Diamond DM, Alkadhi KA (2004) Adverse effect of the combination of hypothyroidism and chronic psychosocial stress on hippocampus-dependent memory in rats. Behavioural Brain Research 155:77-84.

Gerges NZ, Stringer JL, Alkadhi KA (2001) Combination of hypothyroidism and stress abolishes early LTP in the CA1 but not dentate gyrus of hippocampus of adult rats. Brain Research 922:250-260.
Ghosh AK, Kumaragurubaran N, Hong L, Koelsh G, Tang J (2008) Memapsin 2 (beta-secretase) inhibitors: drug development. Current Alzheimer research 5:121-131.
Gibson GE, Sheu KF, Blass JP (1998) Abnormalities of mitochondrial enzymes in Alzheimer disease. Journal of neural transmission 105:855-870.
Gibson GE, Vestling M, Zhang H, Szolosi S, Alkon D, Lannfelt L, Gandy S, Cowburn RF (1997) Abnormalities in Alzheimer's disease fibroblasts bearing the APP670/671 mutation. Neurobiology of aging 18:573-580.
Giovannini MG (2006) The role of the extracellular signal-regulated kinase pathway in memory encoding. Reviews in the neurosciences 17:619-634.
Gold PE (2003) Acetylcholine modulation of neural systems involved in learning and memory. Neurobiology of learning and memory 80:194-210.
Gomez-Pinilla F, Vaynman S, Ying Z (2008) Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. The European journal of neuroscience 28:2278-2287.
Gomez A, Rousset S, Charnallet A (2012) Spatial deficits in an amnesic patient with hippocampal damage: questioning the multiple trace theory. Hippocampus 22:1313-1324.
Gong CX, Lidsky T, Wegiel J, Zuck L, Grundke-Iqbal I, Iqbal K (2000) Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. The Journal of biological chemistry 275:5535-5544.
Goto S, Radak Z, Nyakas C, Chung HY, Naito H, Takahashi R, Nakamoto H, Abea R (2004) Regular exercise: an effective means to reduce oxidative stress in old rats. Annals of the New York Academy of Sciences 1019:471-474.
Gotz J, Ittner LM, Schonrock N, Cappai R (2008) An update on the toxicity of Abeta in Alzheimer's disease. Neuropsychiatric disease and treatment 4:1033-1042.
Gould T, David T. Dao, Kovacsics CE (2009) The open field test. Neuromethods 42:1-20.
Gozal D, Nair D, Goldbart AD (2010) Physical activity attenuates intermittent hypoxia-induced spatial learning deficits and oxidative stress. American journal of respiratory and critical care medicine 182:104-112.
Grace L, Hescham S, Kellaway LA, Bugarith K, Russell VA (2009) Effect of exercise on learning and memory in a rat model of developmental stress. Metab Brain Dis 24:643-657.
Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM (2006) Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:9047-9056.
Griesbach GS, Hovda DA, Gomez-Pinilla F (2009) Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. Brain Research 1288:105-115.
Grimm S (2012) The ER-mitochondria interface: the social network of cell death. Biochimica et biophysica acta 1823:327-334.
Grothe M, Heinsen H, Teipel SJ (2012) Atrophy of the cholinergic Basal forebrain over the adult age range and in early stages of Alzheimer's disease. Biological psychiatry 71:805-813.
Grunwald T, Pezer N, Munte TF, Kurthen M, Lehnertz K, Van Roost D, Fernandez G, Kutas M, Elger CE (2003) Dissecting out conscious and unconscious memory (sub)processes within the human medial temporal lobe. NeuroImage 20 Suppl 1:S139-145.
Gu L, Guo Z (2013) Alzheimer's Abeta42 and Abeta40 peptides form interlaced amyloid fibrils. Journal of neurochemistry.
Gulyas B, Brockschnieder D, Nag S, Pavlova E, Kasa P, Beliczai Z, Legradi A, Gulya K, Thiele A, Dyrks T, Halldin C (2010) The norepinephrine transporter (NET) radioligand (S,S)-[18F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer's disease: a post-mortem autoradiographic study. Neurochemistry international 56:789-798.

Hamann S, Monarch ES, Goldstein FC (2002) Impaired fear conditioning in Alzheimer's disease. Neuropsychologia 40:1187-1195.

Hamer M, Chida Y (2009) Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. Psychological medicine 39:3-11.

Han X (2010) The pathogenic implication of abnormal interaction between apolipoprotein E isoforms, amyloid-beta peptides, and sulfatides in Alzheimer's disease. Molecular neurobiology 41:97-106.

Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. Journal of neurochemistry 110:1129-1134.

Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends in pharmacological sciences 12:383-388.

Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG (2000) Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. Biochemical and biophysical research communications 276:422-427.

Hartmann A, Veldhuis JD, Deuschle M, Standhardt H, Heuser I (1997) Twenty-four hour cortisol release profiles in patients with Alzheimer's and Parkinson's disease compared to normal controls: ultradian secretory pulsatility and diurnal variation. Neurobiology of aging 18:285-289.

Hasegawa S, Furuichi T, Yoshida T, Endoh K, Kato K, Sado M, Maeda R, Kitamoto A, Miyao T, Suzuki R, Homma S, Masushige S, Kajii Y, Kida S (2009) Transgenic up-regulation of alpha-CaMKII in forebrain leads to increased anxiety-like behaviors and aggression. Molecular brain 2:6.

Hauser T, Klaus F, Lipp HP, Amrein I (2009) No effect of running and laboratory housing on adult hippocampal neurogenesis in wild caught long-tailed wood mouse. BMC neuroscience 10:43.

Helfer JL, Goodlett CR, Greenough WT, Klintsova AY (2009) The effects of exercise on adolescent hippocampal neurogenesis in a rat model of binge alcohol exposure during the brain growth spurt. Brain Res 1294:1-11.

Henderson VW, Mack W, Williams BW (1989) Spatial disorientation in Alzheimer's disease. Archives of neurology 46:391-394.

Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, Jardanhazi-Kurutz D, Walter J, Kirchhoff F, Hanisch UK, Kummer MP (2010) Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. Proceedings of the National Academy of Sciences of the United States of America 107:6058-6063.

Hennigan A, Callaghan CK, Kealy J, Rouine J, Kelly AM (2009) Deficits in LTP and recognition memory in the genetically hypertensive rat are associated with decreased expression of neurotrophic factors and their receptors in the dentate gyrus. Behavioural Brain Research 197:371-377.

Herholz K, Weisenbach S, Zundorf G, Lenz O, Schroder H, Bauer B, Kalbe E, Heiss WD (2004) In vivo study of acetylcholine esterase in basal forebrain, amygdala, and cortex in mild to moderate Alzheimer disease. NeuroImage 21:136-143.

Hescham S, Grace L, Kellaway LA, Bugarith K, Russell VA (2009) Effect of exercise on synaptophysin and calcium/calmodulin-dependent protein kinase levels in prefrontal cortex and hippocampus of a rat model of developmental stress. Metabolic brain disease 24:701-709.

Heyn P, Abreu BC, Ottenbacher KJ (2004) The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis. Archives of physical medicine and rehabilitation 85:1694-1704.

Higgins GA, Jacobsen H (2003) Transgenic mouse models of Alzheimer's disease: phenotype and application. Behavioural pharmacology 14:419-438.

Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL (1996) Insulin-like growth factor I of peripheral origin acts centrally to accelerate the initiation of female puberty. Endocrinology 137:3717-3728.

Ho N, Liauw JA, Blaeser F, Wei F, Hanissian S, Muglia LM, Wozniak DF, Nardi A, Arvin KL, Holtzman DM, Linden DJ, Zhuo M, Muglia LJ, Chatila TA (2000) Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:6459-6472.

Ho OH, Delgado JY, O'Dell TJ (2004) Phosphorylation of proteins involved in activity-dependent forms of synaptic plasticity is altered in hippocampal slices maintained in vitro. Journal of neurochemistry 91:1344-1357.

Hock BJ, Lattal KM, Kulnane LS, Abel T, Lamb BT (2009) Pathology associated memory deficits in Swedish mutant genome-based amyloid precursor protein transgenic mice. Current aging science 2:205-213.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nature medicine 4:97-100.

Holsinger RM, McLean CA, Beyreuther K, Masters CL, Evin G (2002) Increased expression of the amyloid precursor beta-secretase in Alzheimer's disease. Annals of neurology 51:783-786.

Hong CJ, Liou YJ, Tsai SJ (2011) Effects of BDNF polymorphisms on brain function and behavior in health and disease. Brain Research bulletin 86:287-297.

Hopkins ME, Bucci DJ (2010) Interpreting the effects of exercise on fear conditioning: the influence of time of day. Behavioral Neuroscience 124:868-872.

Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2006) Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. Journal of neuroscience research 84:1495-1504.

Horsburgh K, Cole GM, Yang F, Savage MJ, Greenberg BD, Gentleman SM, Graham DI, Nicoll JA (2000) beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. Neuropathology and applied neurobiology 26:124-132.

Hosseini N, Alaei H, Reisi P, Radahmadi M (2013) The effect of treadmill running on passive avoidance learning in animal model of Alzheimer disease. International journal of preventive medicine 4:187-192.

Hotting K, Roder B (2013) Beneficial effects of physical exercise on neuroplasticity and cognition. Neuroscience and biobehavioral reviews.

Hoveida R, Alaei H, Oryan S, Parivar K, Reisi P (2011) Treadmill running improves spatial memory in an animal model of Alzheimer's disease. Behavioural Brain Research 216:270-274.

Hsu JC, Zhang Y, Takagi N, Gurd JW, Wallace MC, Zhang L, Eubanks JH (1998) Decreased expression and functionality of NMDA receptor complexes persist in the CA1, but not in the dentate gyrus after transient cerebral ischemia. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 18:768-775.

Huang CH, Chiang YW, Liang KC, Thompson RF, Liu IY (2010) Extra-cellular signal-regulated kinase 1/2 (ERK1/2) activated in the hippocampal CA1 neurons is critical for retrieval of auditory trace fear memory. Brain Research 1326:143-151.

Huang SM, Mouri A, Kokubo H, Nakajima R, Suemoto T, Higuchi M, Staufenbiel M, Noda Y, Yamaguchi H, Nabeshima T, Saido TC, Iwata N (2006) Neprilysin-sensitive synapse-associated amyloid-beta peptide oligomers impair neuronal plasticity and cognitive function. The Journal of biological chemistry 281:17941-17951.

Hunter T, Schulman H (2005) CaMKII structure--an elegant design. Cell 123:765-767.

Hwang IK, Lee KY, Yoo KY, Kim DS, Lee NS, Jeong YG, Kang TC, Han BH, Kim JS, Won MH (2005) Tyrosine kinase A but not phosphacan/protein tyrosine phosphatase-zeta/beta immunoreactivity and protein level changes in neurons and astrocytes in the gerbil hippocampus proper after transient forebrain ischemia. Brain Research 1036:35-41.

Ikeda E, Shiba K, Mori H, Ichikawa A, Sumiya H, Kuji I, Tonami N (2000) Reduction of vesicular acetylcholine transporter in beta-amyloid protein-infused rats with memory impairment. Nuclear medicine communications 21:933-937.

Inestrosa NC, Dinamarca MC, Alvarez A (2008) Amyloid-cholinesterase interactions. Implications for Alzheimer's disease. The FEBS journal 275:625-632.

Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT (1997) APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. Journal of neuropathology and experimental neurology 56:965-973.

Isaacs KR, Anderson BJ, Alcantara AA, Black JE, Greenough WT (1992) Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 12:110-119.

Isomae K, Morimoto S, Hasegawa H, Morita K, Kamei J (2003) Effects of T-82, a novel acetylcholinesterase inhibitor, on impaired learning and memory in passive avoidance task in rats. European journal of pharmacology 465:97-103.

Itoh A, Akaike T, Sokabe M, Nitta A, Iida R, Olariu A, Yamada K, Nabeshima T (1999) Impairments of long-term potentiation in hippocampal slices of beta-amyloid-infused rats. European journal of pharmacology 382:167-175.

Itoh A, Nitta A, Nadai M, Nishimura K, Hirose M, Hasegawa T, Nabeshima T (1996) Dysfunction of cholinergic and dopaminergic neuronal systems in beta-amyloid protein--infused rats. Journal of neurochemistry 66:1113-1117.

Jancic D, Lopez de Armentia M, Valor LM, Olivares R, Barco A (2009) Inhibition of cAMP response element-binding protein reduces neuronal excitability and plasticity, and triggers neurodegeneration. Cerebral cortex 19:2535-2547.

Jang SW, Okada M, Sayeed I, Xiao G, Stein D, Jin P, Ye K (2007) Gambogic amide, a selective agonist for TrkA receptor that possesses robust neurotrophic activity, prevents neuronal cell death. Proceedings of the National Academy of Sciences of the United States of America 104:16329-16334.

Jin J, Jing H, Choi G, Oh MS, Ryu JH, Jeong JW, Huh Y, Park C (2008) Voluntary exercise increases the new cell formation in the hippocampus of ovariectomized mice. Neuroscience letters 439:260-263.

Jin J, Kang HM, Park C (2010) Voluntary exercise enhances survival and migration of neural progenitor cells after intracerebral haemorrhage in mice. Brain injury : [BI] 24:533-540.

Jin N, Qian W, Yin X, Zhang L, Iqbal K, Grundke-Iqbal I, Gong CX, Liu F (2013) CREB regulates the expression of neuronal glucose transporter 3: a possible mechanism related to impaired brain glucose uptake in Alzheimer's disease. Nucleic acids research 41:3240-3256.

Jo J WD, Olsen KM, Kerrigan TL, Lo SC, Bru-Mercier G, Dickinson B, Scullion S, Sheng M, Collingridge G, Cho K (2011) Aβ(1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β. Nature Neuroscience14:545-547.

Johnson LR, Rush JW, Turk JR, Price EM, Laughlin MH (2001) Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. Journal of Applied Physiology 90:1102-1110.

Johnson NA, Jahng GH, Weiner MW, Miller BL, Chui HC, Jagust WJ, Gorno-Tempini ML, Schuff N (2005) Pattern of cerebral hypoperfusion in Alzheimer disease and mild cognitive impairment measured with arterial spin-labeling MR imaging: initial experience. Radiology 234:851-859.

Jost BC, Grossberg GT (1996) The evolution of psychiatric symptoms in Alzheimer's disease: a natural history study. Journal of the American Geriatrics Society 44:1078-1081.

Kamei H, Nagai T, Nakano H, Togan Y, Takayanagi M, Takahashi K, Kobayashi K, Yoshida S, Maeda K, Takuma K, Nabeshima T, Yamada K (2006) Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. Biological Psychiatry 59:75-84.

Kamijo K, Nishihira Y, Hatta A, Kaneda T, Wasaka T, Kida T, Kuroiwa K (2004) Differential influences of exercise intensity on information processing in the central nervous system. European Journal of Applied Physiology 92:305-311.

Kandel ER (2012) The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Molecular Brain 5:14.

Kang H, Sun LD, Atkins CM, Soderling TR, Wilson MA, Tonegawa S (2001) An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell 106:771-783.

Kannangara TS, Lucero MJ, Gil-Mohapel J, Drapala RJ, Simpson JM, Christie BR, van Praag H (2011) Running reduces stress and enhances cell genesis in aged mice. Neurobiology of Aging 32:2279-2286.

Kapay NA, Popova OV, Isaev NK, Stelmashook EV, Kondratenko RV, Zorov DB, Skrebitsky VG, Skulachev VP (2013) Mitochondria-Targeted Plastoquinone Antioxidant SkQ1 Prevents Amyloid-beta-Induced Impairment of Long-Term Potentiation in Rat Hippocampal Slices. Journal of Alzheimer's disease 36:377-383.

Kasahara J, Fukunaga K, Miyamoto E (2001) Activation of calcium/calmodulin-dependent protein kinase IV in long term potentiation in the rat hippocampal CA1 region. The Journal of Biological Chemistry 276:24044-24050.

Kennard JA, Woodruff-Pak DS (2012) A comparison of low- and high-impact forced exercise: effects of training paradigm on learning and memory. Physiology & Behavior 106:423-427.

Kesner RP (2013a) An analysis of the dentate gyrus function. Behavioural Brain Research.

Kesner RP (2013b) A process analysis of the CA3 subregion of the hippocampus. Frontiers in Cellular Neuroscience 7:78.

Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F (1998) Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? Behavioral Neuroscience 112:1012-1019.

Khabour OF, Alzoubi KH, Alomari MA, Alzubi MA (2010) Changes in spatial memory and BDNF expression to concurrent dietary restriction and voluntary exercise. Hippocampus 20:637-645.

Kida S (2012) A Functional Role for CREB as a Positive Regulator of Memory Formation and LTP. Experimental Neurobiology 21:136-140.

Kim H, Lee SH, Kim SS, Yoo JH, Kim CJ (2007) The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. International Journal of Developmental Neuroscience 25:243-249.

Kim SE, Ko IG, Kim BK, Shin MS, Cho S, Kim CJ, Kim SH, Baek SS, Lee EK, Jee YS (2010) Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. Experimental Gerontology 45:357-365.

Kirchner L, Chen WQ, Afjehi-Sadat L, Viidik A, Skalicky M, Hoger H, Lubec G (2008) Hippocampal metabolic proteins are modulated in voluntary and treadmill exercise rats. Experimental neurology 212:145-151.

Kiyosawa M, Baron JC, Hamel E, Pappata S, Duverger D, Riche D, Mazoyer B, Naquet R, MacKenzie ET (1989) Time course of effects of unilateral lesions of the nucleus basalis of Meynert on glucose utilization by the cerebral cortex. Positron tomography in baboons. Brain 112 (Pt 2):435-455.

Kleim JA, Cooper NR, VandenBerg PM (2002) Exercise induces angiogenesis but does not alter movement representations within rat motor cortex. Brain Research 934:1-6.

Kleim JA, Lussnig E, Schwarz ER, Comery TA, Greenough WT (1996) Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. The Journal of Neuroscience 16:4529-4535.

Kline DD, Ogier M, Kunze DL, Katz DM (2010) Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice. The Journal of Neuroscience 30:5303-5310.

Knight EM, Brown TM, Gumusgoz S, Smith JC, Waters EJ, Allan SM, Lawrence CB (2013) Age-related changes in core body temperature and activity in triple-transgenic Alzheimer's disease (3xTgAD) mice. Disease Models & Mechanisms 6:160-170.

Knobloch M, Farinelli M, Konietzko U, Nitsch RM, Mansuy IM (2007) Abeta oligomer-mediated long-term potentiation impairment involves protein phosphatase 1-dependent mechanisms. Journal ofl Neuroscience27:7648-7653.

Koch G, Di Lorenzo F, Bonni S, Ponzo V, Caltagirone C, Martorana A (2012) Impaired LTP- but not LTD-like cortical plasticity in Alzheimer's disease patients. Journal of Alzheimer's Disease 31:593-599.

Kolbeck R, Jungbluth S, Barde YA (1994) Characterisation of neurotrophin dimers and monomers. European Journal of Biochemistry 225:995-1003.

Kraemer R, Baker PJ, Kent KC, Ye Y, Han JJ, Tejada R, Silane M, Upmacis R, Deeb R, Chen Y, Levine DM, Hempstead B (2005) Decreased neurotrophin TrkB receptor expression reduces lesion size in the apolipoprotein E-null mutant mouse. Circulation 112:3644-3653.

Kramar EA, Lin B, Lin CY, Arai AC, Gall CM, Lynch G (2004) A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. The Journal of Neuroscience 24:5151-5161.

Krityakiarana W, Espinosa-Jeffrey A, Ghiani CA, Zhao PM, Topaldjikian N, Gomez-Pinilla F, Yamaguchi M, Kotchabhakdi N, de Vellis J (2010) Voluntary exercise increases oligodendrogenesis in spinal cord. The International Journal of Neuroscience 120:280-290.

Kromer Vogt LJ, Hyman BT, Van Hoesen GW, Damasio AR (1990) Pathological alterations in the amygdala in Alzheimer's disease. Neuroscience 37:377-385.

Krugel U, Bigl V, Eschrich K, Bigl M (2001) Deafferentation of the septo-hippocampal pathway in rats as a model of the metabolic events in Alzheimer's disease. International Journal of Developmental Neuroscience 19:263-277.

Kuchibhotla KV, Goldman ST, Lattarulo CR, Wu HY, Hyman BT, Bacskai BJ (2008) Abeta plaques lead to aberrant regulation of calcium homeostasis in vivo resulting in structural and functional disruption of neuronal networks. Neuron 59:214-225.

Kumar S, Walter J (2011) Phosphorylation of amyloid beta (Abeta) peptides - a trigger for formation of toxic aggregates in Alzheimer's disease. Aging 3:803-812.

Kuret J, Chirita CN, Congdon EE, Kannanayakal T, Li G, Necula M, Yin H, Zhong Q (2005) Pathways of tau fibrillization. Biochimica et Biophysica Acta 1739:167-178.

Lahiri DK (2004) Functional characterization of amyloid beta precursor protein regulatory elements: rationale for the identification of genetic polymorphism. Annals of the New York Academy of Sciences 1030:282-288.

Lau YS, Patki G, Das-Panja K, Le WD, Ahmad SO (2011) Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. The European Journal of Neuroscience 33:1264-1274.

Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, Greenop KR, Almeida OP (2008) Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. The Journal of the American Medical Association 300:1027-1037.

Leal G, Comprido D, Duarte CB (2013) BDNF-induced local protein synthesis and synaptic plasticity. Neuropharmacology.

Leasure JL, Jones M (2008) Forced and voluntary exercise differentially affect brain and behavior. Neuroscience 156:456-465.

Leasure JL, Nixon K (2010) Exercise neuroprotection in a rat model of binge alcohol consumption. Alcoholism, Clinical and Experimental Research 34:404-414.

Lecanu L, Papadopoulos V (2013) Modeling Alzheimer's disease with non-transgenic rat models. Alzheimer's Research & Therapy 5:17.

Lee E, Son H (2009) Adult hippocampal neurogenesis and related neurotrophic factors. BMB Reports 42:239-244.

Lee EB, Leng LZ, Zhang B, Kwong L, Trojanowski JQ, Abel T, Lee VM (2006) Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. The Journal of Biological Chemistry 281:4292-4299.

Lee KH, Chatila TA, Ram RA, Thompson RF (2009) Impaired memory of eyeblink conditioning in CaMKIV KO mice. Behavioral Neuroscience 123:438-442.

Lee KJ, Park IS, Kim H, Greenough WT, Pak DT, Rhyu IJ (2013) Motor Skill Training Induces Coordinated Strengthening and Weakening between Neighboring Synapses. The Journal of Neuroscience 33:9794-9799.

Lemere CA, Masliah E (2010) Can Alzheimer disease be prevented by amyloid-beta immunotherapy? Nature Reviews Neurology 6:108-119.

Lieberman DN, Mody I (1994) Regulation of NMDA channel function by endogenous Ca(2+)-dependent phosphatase. Nature 369:235-239.

Liu J, Kennedy DJ, Yan Y, Shapiro JI (2012) Reactive Oxygen Species Modulation of Na/K-ATPase Regulates Fibrosis and Renal Proximal Tubular Sodium Handling. International Journal of Nephrology 2012:381320.

Ljungberg MC, Ali YO, Zhu J, Wu CS, Oka K, Zhai RG, Lu HC (2012) CREB-activity and nmnat2 transcription are down-regulated prior to neurodegeneration, while NMNAT2 over-expression is neuroprotective, in a mouse model of human tauopathy. Human Molecular Genetics 21:251-267.

Lo AC, Iscru E, Blum D, Tesseur I, Callaerts-Vegh Z, Buee L, De Strooper B, Balschun D, D'Hooge R (2013) Amyloid and Tau Neuropathology Differentially Affect Prefrontal Synaptic Plasticity and Cognitive Performance in Mouse Models of Alzheimer's Disease. Journal of Alzheimer's Disease.

Lommatzsch M, Braun A, Mannsfeldt A, Botchkarev VA, Botchkareva NV, Paus R, Fischer A, Lewin GR, Renz H (1999) Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived Neurotrophic functions. The American Journal of Pathology 155:1183-1193.

Lomo T (2003) The discovery of long-term potentiation. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 358:617-620.

Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605-623.

Lovestone S, Reynolds CH (1997) The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes. Neuroscience 78:309-324.

Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nature Reviews Neuroscience 6:603-614.

Lu FM, Hawkins RD (2006) Presynaptic and postsynaptic Ca(2+) and CamKII contribute to long-term potentiation at synapses between individual CA3 neurons. Proceedings of the National Academy of Sciences of the United States of America 103:4264-4269.

Luo CX, Jiang J, Zhou QG, Zhu XJ, Wang W, Zhang ZJ, Han X, Zhu DY (2007) Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. Journal of Neuroscience Research 85:1637-1646.

Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL, Meaney MJ (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nature Neuroscience1:69-73.

Ly PT, Wu Y, Zou H, Wang R, Zhou W, Kinoshita A, Zhang M, Yang Y, Cai F, Woodgett J, Song W (2013) Inhibition of GSK3beta-mediated BACE1 expression reduces Alzheimer-associated phenotypes. The Journal of Clinical Investigation 123:224-235.

Lynch G, Kessler M, Arai A, Larson J (1990) The nature and causes of hippocampal long-term potentiation. Progress in Brain Research 83:233-250.

Lynch MA, Bliss TV (1986) Noradrenaline modulates the release of [14C]glutamate from dentate but not from CA1/CA3 slices of rat hippocampus. Neuropharmacology 25:493-498.

Lynch WJ, Peterson AB, Sanchez V, Abel J, Smith MA (2013) Exercise as a novel treatment for drug addiction: A neurobiological and stage-dependent hypothesis. Neuroscience and Biobehavioral Reviews.

Ma T, Hoeffer CA, Wong H, Massaad CA, Zhou P, Iadecola C, Murphy MP, Pautler RG, Klann E (2011) Amyloid beta-induced impairments in hippocampal synaptic plasticity are rescued by decreasing mitochondrial superoxide. Journal of Neuroscience Research 31:5589-5595.

Maddox WT, Ashby FG (2004) Dissociating explicit and procedural-learning based systems of perceptual category learning. Behavioural Processes 66:309-332.

Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. Experientia 42:109-114.

Malamut BL, Graff-Radford N, Chawluk J, Grossman RI, Gur RC (1992) Memory in a case of bilateral thalamic infarction. Neurology 42:163-169.

Manich G, Mercader C, del Valle J, Duran-Vilaregut J, Camins A, Pallas M, Vilaplana J, Pelegri C (2011) Characterization of amyloid-beta granules in the hippocampus of SAMP8 mice. Journal of Alzheimer's Disease 25:535-546.

Mao L, Tang Q, Samdani S, Liu Z, Wang JQ (2004) Regulation of MAPK/ERK phosphorylation via ionotropic glutamate receptors in cultured rat striatal neurons. The European Journal of Neuroscience 19:1207-1216.

Marco EM, Valero M, de la Serna O, Aisa B, Borcel E, Ramirez MJ, Viveros MP (2013) Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats. Neuropharmacology 68:223-231.

Martin-Rehrmann MD, Hoe HS, Capuani EM, Rebeck GW (2005) Association of apolipoprotein J-positive beta-amyloid plaques with dystrophic neurites in Alzheimer's disease brain. Neurotoxicity Research 7:231-242.

Maruszak A, Zekanowski C (2011) Mitochondrial dysfunction and Alzheimer's disease. Progress in Neuro-psychopharmacology & Biological Psychiatry 35:320-330.

Massey KA, Zago WM, Berg DK (2006) BDNF up-regulates alpha7 nicotinic acetylcholine receptor levels on subpopulations of hippocampal interneurons. Molecular and Cellular Neurosciences 33:381-388.

Matsuda H, Koyama H, Oikawa M, Yoshihara T, Kaneko M (1991) Nerve growth factor-like activity detected in equine peripheral blood after running exercise. Zentralblatt fur Veterinarmedizin Reihe A 38:557-559.

Matsuo N, Yamasaki N, Ohira K, Takao K, Toyama K, Eguchi M, Yamaguchi S, Miyakawa T (2009) Neural activity changes underlying the working memory deficit in alpha-CaMKII heterozygous knockout mice. Frontiers in Behavioral Neuroscience 3:20.

Mattei TA (2013) Is it all about Contact? Neurodegeneration as a "Protein Freeze Tag Game" Inside the Central Nervous System. Frontiers in Neurology 4:75.

McCullough MJ, Gyorkos AM, Spitsbergen JM (2013) Short-term exercise increases GDNF protein levels in the spinal cord of young and old rats. Neuroscience 240:258-268.

McLaren AN, Lamantia MA, Callahan CM (2013) Systematic review of non-pharmacologic interventions to delay functional decline in community-dwelling patients with dementia. Aging & Mental Health.

Mega MS, Cummings JL, Fiorello T, Gornbein J (1996) The spectrum of behavioral changes in Alzheimer's disease. Neurology 46:130-135.

Mellor J, Nicoll R (2001) Hippocampal mossy fiber LTP is independent of postsynaptic calcium. Nature Neuroscience 4:125-126.

Middei S, Houeland G, Cavallucci V, Ammassari-Teule M, D'Amelio M, Marie H (2013) CREB is necessary for synaptic maintenance and learning-induced changes of the ampa receptor GluA1 subunit. Hippocampus 23:488-499.

Miller LJ (2007) The use of cognitive enhancers in behavioral disturbances of Alzheimer's disease. The Consultant pharmacist 22:754-762.

Miners JS, Barua N, Kehoe PG, Gill S, Love S (2011) Abeta-degrading enzymes: potential for treatment of Alzheimer disease. Journal of Neuropathology and Experimental Neurology 70:944-959.

Mohajeri MH, Saini KD, Nitsch RM (2004) Transgenic BACE expression in mouse neurons accelerates amyloid plaque pathology. Journal of Neural Transmission 111:413-425.

Mohapel P, Leanza G, Kokaia M, Lindvall O (2005) Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. Neurobiology of Aging 26:939-946.

Monzon-Mayor M, Alvarez M, Arbelo-Galvan J, Romero-Aleman M, Yanes C, Plaza ML, Rodriguez JR, Rodriguez JJ, Toledano A (2000) Long-term evolution of local, proximal and remote astrocyte responses after diverse nucleus basalis lesioning (an experimental Alzheimer model): GFAP immunocytochemical study. Brain Research 865:245-258.

Moore MJ, Zhu CW, Clipp EC (2001) Informal costs of dementia care: estimates from the National Longitudinal Caregiver Study. The Journals of Gerontology Series B, Psychological Sciences and Social sciences 56:S219-228.

Morgan D (2011) Immunotherapy for Alzheimer's disease. Journal of Internal Medicine 269:54-63.

Morris JC, Storandt M, McKeel DW, Jr., Rubin EH, Price JL, Grant EA, Berg L (1996) Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology 46:707-719.

Morris RG (1989) Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. The Journal of Neuroscience 9:3040-3057.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683.

Morris RG, Schenk F, Tweedie F, Jarrard LE (1990) Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning. The European Journal of Neuroscience 2:1016-1028.

Morris RG, Steele RJ, Bell JE, Martin SJ (2013) N-methyl-d-aspartate receptors, learning and memory: chronic intraventricular infusion of the NMDA receptor antagonist d-AP5 interacts directly with the neural mechanisms of spatial learning. The European Journal of Neuroscience 37:700-717.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8:608-619.

Mueller SG, Schuff N, Yaffe K, Madison C, Miller B, Weiner MW (2010) Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. Human Brain Mapping 31:1339-1347.

Mufson EJ, Ginsberg SD, Ikonomovic MD, DeKosky ST (2003) Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. Journal of Chemical Neuroanatomy 26:233-242.

Mukhamedyarov MA, Kochunova JO, Yusupova ER, Haidarov BA, Zefirov AL, Palotas A (2010) The contribution of calcium/calmodulin-dependent protein-kinase II (CaMKII) to short-term plasticity at the neuromuscular junction. Brain Research Bulletin 81:613-616.

Mukherjee A, Hersh LB (2002) Regulation of amyloid beta-peptide levels by enzymatic degradation. Journal of Alzheimer's disease : JAD 4:341-348.

Murray CL, Fibiger HC (1986) Pilocarpine and physostigmine attenuate spatial memory impairments produced by lesions of the nucleus basalis magnocellularis. Behavioral Neuroscience 100:23-32.

Nag S, Tang F, Yee BK (2001) Chronic intracerebroventricular exposure to beta-amyloid(1-40) impairs object recognition but does not affect spontaneous locomotor activity or sensorimotor gating in the rat. Experimental Brain Research 136:93-100.

Nakajima S, Ohsawa I, Ohta S, Ohno M, Mikami T (2010) Regular voluntary exercise cures stress-induced impairment of cognitive function and cell proliferation accompanied by increases in cerebral IGF-1 and GST activity in mice. Behavioural Brain Research 211:178-184.

Nakamura S, Murayama N, Noshita T, Annoura H, Ohno T (2001) Progressive brain dysfunction following intracerebroventricular infusion of beta(1-42)-amyloid peptide. Brain Research 912:128-136.

Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J, Goncalves O, Verge G, Momoli F, Welner SA, Massicotte G, Julien JP, Shapiro ML (1997) Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. Nature 387:500-505.

Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. The Journal of the American Medical Association 283:1571-1577.

Nayak A, Browning MD (1999) Presynaptic and postsynaptic mechanisms of long-term potentiation. Advances in Neurology 79:645-658.

Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Research 726:49-56.

Nichol K, Deeny SP, Seif J, Camaclang K, Cotman CW (2009) Exercise improves cognition and hippocampal plasticity in APOE epsilon4 mice. Alzheimer's & Dementia 5:287-294.

Nichol KE, Parachikova AI, Cotman CW (2007) Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. Behavioural Brain Research 184:124-132.

Nicoll RA (2003) Expression mechanisms underlying long-term potentiation: a postsynaptic view. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 358:721-726.

Niewoehner B, Single FN, Hvalby O, Jensen V, Meyer zum Alten Borgloh S, Seeburg PH, Rawlins JN, Sprengel R, Bannerman DM (2007) Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. The European Journal of Neuroscience 25:837-846.

Nilsson L, Nordberg A, Hardy J, Wester P, Winblad B (1986) Physostigmine restores 3H-acetylcholine efflux from Alzheimer brain slices to normal level. Journal of Neural Transmission 67:275-285.

Nistico R, Pignatelli M, Piccinin S, Mercuri NB, Collingridge G (2012) Targeting synaptic dysfunction in Alzheimer's disease therapy. Molecular Neurobiology 46:572-587.

Nitta A, Fukuta T, Hasegawa T, Nabeshima T (1997) Continuous infusion of beta-amyloid protein into the rat cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Japanese Journal of Pharmacology 73:51-57.

Nitta A, Itoh A, Hasegawa T, Nabeshima T (1994) beta-Amyloid protein-induced Alzheimer's disease animal model. Neuroscience Letters 170:63-66.

O'Callaghan RM, Griffin EW, Kelly AM (2009) Long-term treadmill exposure protects against age-related neurodegenerative change in the rat hippocampus. Hippocampus 19:1019-1029.

O'Callaghan RM, Ohle R, Kelly AM (2007) The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. Behavioural Brain Research 176:362-366.

O'Keefe J (1976) Place units in the hippocampus of the freely moving rat. Experimental Neurology 51:78-109.

Ogonovszky H, Berkes I, Kumagai S, Kaneko T, Tahara S, Goto S, Radak Z (2005) The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. Neurochemistry International 46:635-640.

Okada T, Yamada N, Tsuzuki K, Horikawa HP, Tanaka K, Ozawa S (2003) Long-term potentiation in the hippocampal CA1 area and dentate gyrus plays different roles in spatial learning. The European Journal of Neuroscience 17:341-349.

Okano H, Hirano T, Balaban E (2000) Learning and memory. Proceedings of the National Academy of Sciences 97:12403-12404.

Olton DS (1990) Dementia: animal models of the cognitive impairments following damage to the basal forebrain cholinergic system. Brain Research Bulletin 25:499-502.

Olton DS, Walker JA, Gage FH (1978) Hippocampal connections and spatial discrimination. Brain Research 139:295-308.

Ozkaya YG, Agar A, Yargicoglu P, Hacioglu G, Bilmen-Sarikcioglu S, Ozen I, Aliciguzel Y (2002) The effect of exercise on brain antioxidant status of diabetic rats. Diabetes & Metabolism 28:377-384.

Padurariu M, Ciobica A, Mavroudis I, Fotiou D, Baloyannis S (2012) Hippocampal neuronal loss in the CA1 and CA3 areas of Alzheimer's disease patients. Psychiatria Danubina 24:152-158.

Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ (1998) Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 37:1553-1561.

Panza F, Frisardi V, Imbimbo BP, Capurso C, Logroscino G, Sancarlo D, Seripa D, Vendemiale G, Pilotto A, Solfrizzi V (2010) REVIEW: gamma-Secretase inhibitors for the treatment of Alzheimer's disease: The current state. CNS Neuroscience & Therapeutics 16:272-284.

Papatheodoropoulos C, Kostopoulos G (2000) Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. Neuroscience Letters 279:177-180.

Park H, Kam TI, Kim Y, Choi H, Gwon Y, Kim C, Koh JY, Jung YK (2012) Neuropathogenic role of adenylate kinase-1 in Abeta-mediated tau phosphorylation via AMPK and GSK3beta. Human Molecular Genetics 21:2725-2737.

Parvathy S, Davies P, Haroutunian V, Purohit DP, Davis KL, Mohs RC, Park H, Moran TM, Chan JY, Buxbaum JD (2001) Correlation between Abetax-40-, Abetax-42-, and Abetax-43-containing amyloid plaques and cognitive decline. Archives of Neurology 58:2025-2032.

Pedersen BK (2011) Muscles and their myokines. The Journal of Experimental Biology 214:337-346.

Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP (2007) Role of myokines in exercise and metabolism. Journal of Applied Physiology 103:1093-1098.

Pedersen BK, Pedersen M, Krabbe KS, Bruunsgaard H, Matthews VB, Febbraio MA (2009) Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals. Experimentla Physiology 94:1153-1160.

Peers C, Pearson HA, Boyle JP (2007) Hypoxia and Alzheimer's disease. Essays in Biochemistry 43:153-164.

Peng S, Zhang Y, Zhang J, Wang H, Ren B (2010) ERK in learning and memory: A review of recent research. International Journal of Molecular Sciences 11:222-232.

Pergola G, Gunturkun O, Koch B, Schwarz M, Daum I, Suchan B (2012) Recall deficits in stroke patients with thalamic lesions covary with damage to the parvocellular mediodorsal nucleus of the thalamus. Neuropsychologia 50:2477-2491.

Perrin RJ, Fagan AM, Holtzman DM (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature 461:916-922.

Perry EK (1980) The cholinergic system in old age and Alzheimer's disease. Age and ageing 9:1-8.

Pevalova M, Filipcik P, Novak M, Avila J, Iqbal K (2006) Post-translational modifications of tau protein. Bratislavske Lekarske Listy 107:346-353.

Pietrelli A, Lopez-Costa J, Goni R, Brusco A, Basso N (2012) Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience 202:252-266.

Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R (2008) Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis. Oncogene 27:6407-6418.

Pittenger C, Kandel E (1998) A genetic switch for long-term memory. Comptes rendus de l'Academie des sciences Serie III, Sciences de la vie 321:91-96.

Place R, Lykken C, Beer Z, Suh J, McHugh TJ, Tonegawa S, Eichenbaum H, Sauvage MM (2012) NMDA signaling in CA1 mediates selectively the spatial component of episodic memory. Learning & Memory 19:164-169.

Ploughman M (2008) Exercise is brain food: the effects of physical activity on cognitive function. Developmental Neurorehabilitation 11:236-240.

Podlisny MB, Stephenson DT, Frosch MP, Lieberburg I, Clemens JA, Selkoe DJ (1992) Synthetic amyloid beta-protein fails to produce specific neurotoxicity in monkey cerebral cortex. Neurobiology of Aging 13:561-567.

Poduslo JF, Curran GL (1996) Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. Molecular Brain Research 36:280-286.

Potvin O, Allen K, Thibaudeau G, Dore FY, Goulet S (2006) Performance on spatial working memory tasks after dorsal or ventral hippocampal lesions and adjacent damage to the subiculum. Behavioral Neuroscience 120:413-422.

Poulin SP, Dautoff R, Morris JC, Barrett LF, Dickerson BC, Alzheimer's Disease Neuroimaging I (2011) Amygdala atrophy is prominent in early Alzheimer's disease and relates to symptom severity. Psychiatry Research 194:7-13.

Power AE, McIntyre CK, Litmanovich A, McGaugh JL (2003) Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors. Behavioural Pharmacology 14:207-213.

Price DL, Sisodia SS (1998) Mutant genes in familial Alzheimer's disease and transgenic models. Annual Review of Neuroscience 21:479-505.

Pugazhenthi S, Wang M, Pham S, Sze CI, Eckman CB (2011) Downregulation of CREB expression in Alzheimer's brain and in Abeta-treated rat hippocampal neurons. Molecular Neurodegeneration 6:60.

Ra SM, Kim H, Jang MH, Shin MC, Lee TH, Lim BV, Kim CJ, Kim EH, Kim KM, Kim SS (2002) Treadmill running and swimming increase cell proliferation in the hippocampal dentate gyrus of rats. Neuroscience Letters 333:123-126.

Radak Z, Chung HY, Goto S (2005) Exercise and hormesis: oxidative stress-related adaptation for successful aging. Biogerontology 6:71-75.

Radak Z, Toldy A, Szabo Z, Siamilis S, Nyakas C, Silye G, Jakus J, Goto S (2006) The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochemistry International 49:387-392.

Ramirez-Amaya V, Escobar ML, Chao V, Bermudez-Rattoni F (1999) Synaptogenesis of mossy fibers induced by spatial water maze overtraining. Hippocampus 9:631-636.

Ran I, Laplante I, Lacaille JC (2012) CREB-dependent transcriptional control and quantal changes in persistent long-term potentiation in hippocampal interneurons. The Journal of Neuroscience 32:6335-6350.

Raudino F (2013) Non-cognitive symptoms and related conditions in the Alzheimer's disease: a literature review. Neurological Sciences.

Reddy KV, Anuradha D, Kumar TC, Reddanna P (1995) Induction of Ya1 subunit of rat hepatic glutathione S-transferases by exercise-induced oxidative stress. Archives of Biochemistry and Biophysics 323:6-10.

Redila VA, Christie BR (2006) Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus. Neuroscience 137:1299-1307.

Reese LC, Taglialatela G (2011) A role for calcineurin in Alzheimer's disease. Current Neuropharmacology 9:685-692.

Regehr WG, Tank DW (1990) Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. Nature 345:807-810.

Reilly JF, Games D, Rydel RE, Freedman S, Schenk D, Young WG, Morrison JH, Bloom FE (2003) Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model. Proceedings of the National Academy of Sciences of the United States of America 100:4837-4842.

Reisi P, Alaei H, Babri S, Sharifi MR, Mohaddes G (2009) Effects of treadmill running on spatial learning and memory in streptozotocin-induced diabetic rats. Neuroscience Letters 455:79-83.

Reisi P, Babri S, Alaei H, Sharifi MR, Mohaddes G, Noorbakhsh SM, Lashgari R (2010) Treadmill running improves long-term potentiation (LTP) defects in streptozotocin-induced diabetes at dentate gyrus in rats. Pathophysiology 17:33-38.

Reitz C, Honig L, Vonsattel JP, Tang MX, Mayeux R (2009) Memory performance is related to amyloid and tau pathology in the hippocampus. Journal of Neurology, Neurosurgery, and Psychiatry 80:715-721.

Rex CS, Lauterborn JC, Lin CY, Kramar EA, Rogers GA, Gall CM, Lynch G (2006) Restoration of long-term potentiation in middle-aged hippocampus after induction of brain-derived neurotrophic factor. Journal of Neurophysiology 96:677-685.

Rhyu IJ, Bytheway JA, Kohler SJ, Lange H, Lee KJ, Boklewski J, McCormick K, Williams NI, Stanton GB, Greenough WT, Cameron JL (2010) Effects of aerobic exercise training on cognitive function and cortical vascularity in monkeys. Neuroscience 167:1239-1248.

Ridgel AL, Vitek JL, Alberts JL (2009) Forced, not voluntary, exercise improves motor function in Parkinson's disease patients. Neurorehabilitation and Neural Repair 23:600-608.

Rietjens SJ, Beelen M, Koopman R, LJ VANL, Bast A, Haenen GR (2007) A single session of resistance exercise induces oxidative damage in untrained men. Medicine and Science in Sports and Exercise 39:2145-2151.

Rodriguez-Puertas R, Pazos A, Zarranz JJ, Pascual J (1994) Selective cortical decrease of high-affinity choline uptake carrier in Alzheimer's disease: an autoradiographic study using 3H-hemicholinium-3. Journal of Neural Transmission, Parkinson's disease and dementia section 8:161-169.

Rolland Y, Abellan van Kan G, Vellas B (2010) Healthy brain aging: role of exercise and physical activity. Clinics in Geriatric Medicine 26:75-87.

Rolland Y, Pillard F, Klapouszczak A, Reynish E, Thomas D, Andrieu S, Riviere D, Vellas B (2007) Exercise program for nursing home residents with Alzheimer's disease: a 1-year randomized, controlled trial. Journal of the American Geriatrics Society 55:158-165.

Rondi-Reig L, Libbey M, Eichenbaum H, Tonegawa S (2001) CA1-specific N-methyl-D-aspartate receptor knockout mice are deficient in solving a nonspatial transverse patterning task. Proceedings of the National Academy of Sciences of the United States of America 98:3543-3548.

Rosales-Corral S, Acuna-Castroviejo D, Tan DX, Lopez-Armas G, Cruz-Ramos J, Munoz R, Melnikov VG, Manchester LC, Reiter RJ (2012) Accumulation of exogenous amyloid-beta peptide in hippocampal mitochondria causes their dysfunction: a protective role for melatonin. Oxidative Medicine and Cellular Longevity 2012:843649.

Rose AJ, Frosig C, Kiens B, Wojtaszewski JF, Richter EA (2007) Effect of endurance exercise training on Ca2+ calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans. The Journal of Physiology 583:785-795.

Roskoski R, Jr. (2012) ERK1/2 MAP kinases: structure, function, and regulation. Pharmacological Research 66:105-143.

Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. The European Journal of Neuroscience 24:1850-1856.

Rumi-Masante J, Rusinga FI, Lester TE, Dunlap TB, Williams TD, Dunker AK, Weis DD, Creamer TP (2012) Structural basis for activation of calcineurin by calmodulin. Journal of Molecular Biology 415:307-317.

Rylett RJ, Ball MJ, Colhoun EH (1983) Evidence for high affinity choline transport in synaptosomes prepared from hippocampus and neocortex of patients with Alzheimer's disease. Brain Research 289:169-175.

Saarelainen T, Pussinen R, Koponen E, Alhonen L, Wong G, Sirvio J, Castren E (2000) Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons have impaired long-term spatial memory but normal hippocampal LTP. Synapse 38:102-104.

Sabbagh JJ, Kinney JW, Cummings JL (2013) Alzheimer's disease biomarkers: Correspondence between human studies and animal models. Neurobiology of Disease 56:116-130.

Sadigh-Eteghad S, Talebi M, Farhoudi M (2012) Association of apolipoprotein E epsilon 4 allele with sporadic late onset Alzheimer`s disease. A meta-analysis. Neurosciences 17:321-326.

Saint-Cyr JA, Taylor AE, Lang AE (1988) Procedural learning and neostriatal dysfunction in man. Brain 111 (Pt 4):941-959.

Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K (2010) Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. Brain Research 1359:178-185.

Sambamurti K, Kinsey R, Maloney B, Ge YW, Lahiri DK (2004) Gene structure and organization of the human beta-secretase (BACE) promoter. Journal of the Federation of American Societies for Experimental Biology 18:1034-1036.

Sanhueza M, Lisman J (2013) The CaMKII/NMDAR complex as a molecular memory. Molecular brain 6:10.

Santa-Maria I, Varghese M, Ksiezak-Reding H, Dzhun A, Wang J, Pasinetti GM (2012) Paired helical filaments from Alzheimer disease brain induce intracellular accumulation of Tau protein in aggresomes. The Journal of Biological Chemistry 287:20522-20533.

Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, Zhu X (2010) A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. Journal of Alzheimer's Disease 20 Suppl 2:S401-412.

Sawada M, Sawamoto K (2013) Mechanisms of neurogenesis in the normal and injured adult brain. The Keio Journal of Medicine 62:13-28.

Schapiro MB, Rapoport SI (1989) Familial Alzheimer's disease: genetic analysis related to disease heterogeneity, Down syndrome and human brain evolution. Neurobiology of Aging 10:435-436; discussion 446-438.

Schoenfeld TJ, Rada P, Pieruzzini PR, Hsueh B, Gould E (2013) Physical exercise prevents stress-induced activation of granule neurons and enhances local inhibitory mechanisms in the dentate gyrus. The Journal of Neuroscience 33:7770-7777.

Schor NF (2011) What the halted phase III gamma-secretase inhibitor trial may (or may not) be telling us. Annals of Neurology 69:237-239.

Sciolino NR, Dishman RK, Holmes PV (2012) Voluntary exercise offers anxiolytic potential and amplifies galanin gene expression in the locus coeruleus of the rat. Behavioural Brain Research 233:191-200.

Scott SA, DeKosky ST, Sparks DL, Knox CA, Scheff SW (1992) Amygdala cell loss and atrophy in Alzheimer's disease. Annals of Neurology 32:555-563.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery, and Psychiatry 20:11-21.

Sepulcre J, Sabuncu MR, Becker A, Sperling R, Johnson KA (2013) In vivo characterization of the early states of the amyloid-beta network. Brain 136:2239-2252.

Serrano-Pozo A, Muzikansky A, Gomez-Isla T, Growdon JH, Betensky RA, Frosch MP, Hyman BT (2013) Differential relationships of reactive astrocytes and microglia to fibrillar amyloid deposits in Alzheimer disease. Journal of Neuropathology and Experimental Neurology 72:462-471.

Setlow B, McGaugh JL (1999) Involvement of the posteroventral caudate-putamen in memory consolidation in the Morris water maze. Neurobiology of Learning and Memory 71:240-247.

Seubert P, Oltersdorf T, Lee MG, Barbour R, Blomquist C, Davis DL, Bryant K, Fritz LC, Galasko D, Thal LJ, et al. (1993) Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. Nature 361:260-263.

Shah R (2013) The role of nutrition and diet in Alzheimer disease: a systematic review. Journal of the American Medical Directors Association 14:398-402.

Shaw KN, Commins S, O'Mara SM (2003) Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor. The European Journal of Neuroscience 17:2438-2446.

Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annual Review of Biochemistry 68:821-861.

Shen H, Tong L, Balazs R, Cotman CW (2001) Physical activity elicits sustained activation of the cyclic AMP response element-binding protein and mitogen-activated protein kinase in the rat hippocampus. Neuroscience 107:219-229.

Shen J, Maruyama IN (2012) Brain-derived neurotrophic factor receptor TrkB exists as a preformed dimer in living cells. Journal of Solecular signaling 7:2.

Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, Zhu X (2012) Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. Journal of Neurochemistry 120:419-429.

Shimada H, Ataka S, Tomiyama T, Takechi H, Mori H, Miki T (2011) Clinical course of patients with familial early-onset Alzheimer's disease potentially lacking senile plaques bearing the E693Delta mutation in amyloid precursor protein. Dementia and Geriatric Cognitive Disorders 32:45-54.

Shin LM, Liberzon I (2010) The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology 35:169-191.

Shirakura M, Inoue M, Fujikawa S, Washizawa K, Komaba S, Maeda M, Watabe K, Yoshikawa Y, Hasegawa M (2004) Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils. Gene Therapy 11:784-790.

Shruster A, Eldar-Finkelman H, Melamed E, Offen D (2011) Wnt signaling pathway overcomes the disruption of neuronal differentiation of neural progenitor cells induced by oligomeric amyloid beta-peptide. Journal of Neurochemistry 116:522-529.

Shukitt-Hale B, McEwen JJ, Szprengiel A, Joseph JA (2004) Effect of age on the radial arm water maze-a test of spatial learning and memory. Neurobiology of Aging 25:223-229.

Silhol M, Arancibia S, Maurice T, Tapia-Arancibia L (2007) Spatial memory training modifies the expression of brain-derived neurotrophic factor tyrosine kinase receptors in young and aged rats. Neuroscience 146:962-973.

Silman I, Sussman JL (2005) Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Current Opinion in Pharmacology 5:293-302.

Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257:206-211.

Smith JC, Paulson ES, Cook DB, Verber MD, Tian Q (2010) Detecting changes in human cerebral blood flow after acute exercise using arterial spin labeling: implications for fMRI. Journal of Neuroscience Methods 191:258-262.

Smolen P, Baxter DA, Byrne JH (2006) A model of the roles of essential kinases in the induction and expression of late long-term potentiation. Biophysical Journal 90:2760-2775.

Snyder JS, Glover LR, Sanzone KM, Kamhi JF, Cameron HA (2009) The effects of exercise and stress on the survival and maturation of adult-generated granule cells. Hippocampus 19:898-906.

Sofi F, Valecchi D, Bacci D, Abbate R, Gensini GF, Casini A, Macchi C (2011) Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. Journal of Internal Medicine 269:107-117.

Soininen H, Kosunen O, Helisalmi S, Mannermaa A, Paljarvi L, Talasniemi S, Ryynanen M, Riekkinen P, Sr. (1995) A severe loss of choline acetyltransferase in the frontal cortex of Alzheimer patients carrying apolipoprotein epsilon 4 allele. Neuroscience Letters 187:79-82.

Somani SM, Ravi R, Rybak LP (1995) Effect of exercise training on antioxidant system in brain regions of rat. Pharmacology, Biochemistry, and Behavior 50:635-639.

Song D, Wang Z, Berger TW (2002) Contribution of T-type VDCC to TEA-induced long-term synaptic modification in hippocampal CA1 and dentate gyrus. Hippocampus 12:689-697.

Song D, Xie X, Wang Z, Berger TW (2001) Differential effect of TEA on long-term synaptic modification in hippocampal CA1 and dentate gyrus in vitro. Neurobiology of Learning and Memory 76:375-387.

Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, Dietrich A (2003) Exercise activates the endocannabinoid system. Neuroreport 14:2209-2211.

Spencer TK, Mellado W, Filbin MT (2008) BDNF activates CaMKIV and PKA in parallel to block MAG-mediated inhibition of neurite outgrowth. Molecular and Cellular Neuroscience 38:110-116.

Squire LR, Zola-Morgan S, Chen KS (1988) Human amnesia and animal models of amnesia: performance of amnesic patients on tests designed for the monkey. Behavioral Neuroscience 102:210-221.

Srivareerat M, Tran TT, Alzoubi KH, Alkadhi KA (2009) Chronic psychosocial stress exacerbates impairment of cognition and long-term potentiation in beta-amyloid rat model of Alzheimer's disease. Biological Psychiatry 65:918-926.

Srivareerat M, Tran TT, Salim S, Aleisa AM, Alkadhi KA (2011) Chronic nicotine restores normal Abeta levels and prevents short-term memory and E-LTP impairment in Abeta rat model of Alzheimer's disease. Neurobiology of Aging 32:834-844.

Stabel J, Ficker E, Heinemann U (1992) Young CA1 pyramidal cells of rats, but not dentate gyrus granule cells, express a delayed inward rectifying current with properties of IQ. Neuroscience Letters 135:231-234.

Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, Pedersen BK (2001) Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. Journal of Physiology 537:633-639.

Steiner JL, Murphy EA, McClellan JL, Carmichael MD, Davis JM (2011) Exercise training increases mitochondrial biogenesis in the brain. Journal of Applied Physiology 111:1066-1071.

Stepanichev MY, Moiseeva YV, Lazareva NA, Onufriev MV, Gulyaeva NV (2003) Single intracerebroventricular administration of amyloid-beta (25-35) peptide induces impairment in short-term rather than long-term memory in rats. Brain Research Bulletin 61:197-205.

Stephan A, Laroche S, Davis S (2001) Generation of aggregated beta-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. The Journal of Neuroscience 21:5703-5714.

Stephan A, Phillips AG (2005) A case for a non-transgenic animal model of Alzheimer's disease. Genes, Brain, and Behavior 4:157-172.

Stevens CF, Tonegawa S, Wang Y (1994) The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. Current Biology 4:687-693.

Stranahan AM, Lee K, Martin B, Maudsley S, Golden E, Cutler RG, Mattson MP (2009) Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. Hippocampus 19:951-961.

Strong R, Huang JS, Huang SS, Chung HD, Hale C, Burke WJ (1991) Degeneration of the cholinergic innervation of the locus ceruleus in Alzheimer's disease. Brain Research 542:23-28.

Sultana R, Perluigi M, Butterfield DA (2006) Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. Antioxidants & Redox Signaling 8:2021-2037.

Sutherland RJ, McDonald RJ, Hill CR, Rudy JW (1989) Damage to the hippocampal formation in rats selectively impairs the ability to learn cue relationships. Behavioral and Neural Biology 52:331-356.

Suzuki E, Okada T (2007) Regional differences in GABAergic modulation for TEA-induced synaptic plasticity in rat hippocampal CA1, CA3 and dentate gyrus. Neuroscience Research 59:183-190.

Swain RA, Harris AB, Wiener EC, Dutka MV, Morris HD, Theien BE, Konda S, Engberg K, Lauterbur PC, Greenough WT (2003) Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. Neuroscience 117:1037-1046.

Swerdlow RH, Burns JM, Khan SM (2010) The Alzheimer's disease mitochondrial cascade hypothesis. Journal of Alzheimer's Disease 20 Suppl 2:S265-279.

Swerdlow RH, Khan SM (2004) A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. Medical Hypotheses 63:8-20.

Szabo Z, Ying Z, Radak Z, Gomez-Pinilla F (2010) Voluntary exercise may engage proteasome function to benefit the brain after trauma. Brain Research 1341:25-31.

Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, Wang F, Baba T, Tayra JT, Morimoto T, Jing M, Kikuchi Y, Kuramoto S, Agari T, Miyoshi Y, Fujino H, Obata F, Takeda I, Furuta T, Date I (2010) Exercise exerts neuroprotective effects on Parkinson's disease model of rats. Brain Research 1310:200-207.

Tales A, Snowden RJ, Haworth J, Wilcock G (2005) Abnormal spatial and non-spatial cueing effects in mild cognitive impairment and Alzheimer's disease. Neurocase 11:85-92.

Tamagnini F, Burattini C, Casoli T, Balietti M, Fattoretti P, Aicardi G (2012) Early impairment of long-term depression in the perirhinal cortex of a mouse model of Alzheimer's disease. Rejuvenation Research 15:231-234.

Tang K, Xia FC, Wagner PD, Breen EC (2010) Exercise-induced VEGF transcriptional activation in brain, lung and skeletal muscle. Respiratory Physiology & Neurobiology 170:16-22.

Tanzi RE, Kovacs DM, Kim TW, Moir RD, Guenette SY, Wasco W (1996) The gene defects responsible for familial Alzheimer's disease. Neurobiology of disease 3:159-168.

Teipel SJ, Flatz WH, Heinsen H, Bokde AL, Schoenberg SO, Stockel S, Dietrich O, Reiser MF, Moller HJ, Hampel H (2005) Measurement of basal forebrain atrophy in Alzheimer's disease using MRI. Brain 128:2626-2644.

Teixeira AM, Reckziegel P, Muller L, Pereira RP, Roos DH, Rocha JB, Burger ME (2009) Intense exercise potentiates oxidative stress in striatum of reserpine-treated animals. Pharmacology, Biochemistry, and Behavior 92:231-235.

Tejani-Butt SM, Yang J, Zaffar H (1993) Norepinephrine transporter sites are decreased in the locus coeruleus in Alzheimer's disease. Brain Research 631:147-150.

Terwel D, Dewachter I, Van Leuven F (2002) Axonal transport, tau protein, and neurodegeneration in Alzheimer's disease. Neuromolecular Medicine 2:151-165.

Thune-Boyle IC, Iliffe S, Cerga-Pashoja A, Lowery D, Warner J (2012) The effect of exercise on behavioral and psychological symptoms of dementia: towards a research agenda. International Psychogeriatrics 24:1046-1057.

Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M, Racagni G, Popoli M (2004) Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. Neuropsychopharmacology 29:1831-1840.

Toldy A, Atalay M, Stadler K, Sasvari M, Jakus J, Jung KJ, Chung HY, Nyakas C, Radak Z (2009) The beneficial effects of nettle supplementation and exercise on brain lesion and memory in rat. The Journal of Nutritional Biochemistry 20:974-981.

Toledano A, Alvarez MI (2004) Lesions and dysfunctions of the nucleus basalis as Alzheimer's disease models: general and critical overview and analysis of the long-term changes in several excitotoxic models. Current Alzheimer Research 1:189-214.

Tomobe K, Nomura Y (2009) Neurochemistry, neuropathology, and heredity in SAMP8: a mouse model of senescence. Neurochemical Research 34:660-669.

Tong G, Shepherd D, Jahr CE (1995) Synaptic desensitization of NMDA receptors by calcineurin. Science 267:1510-1512.

Toscano-Silva M, Gomes da Silva S, Scorza FA, Bonvent JJ, Cavalheiro EA, Arida RM (2010) Hippocampal mossy fiber sprouting induced by forced and voluntary physical exercise. Physiology & Behavior 101:302-308.

Townsend M, Qu Y, Gray A, Wu Z, Seto T, Hutton M, Shearman MS, Middleton RE (2010) Oral treatment with a gamma-secretase inhibitor improves long-term potentiation in a mouse model of Alzheimer's disease. The Journal of Pharmacology and Experimental Therapeutics 333:110-119.

Tran TT, Srivareerat M, Alkadhi KA (2010) Chronic psychosocial stress triggers cognitive impairment in a novel at-risk model of Alzheimer's disease. Neurobiology of Disease 37:756-763.

Tran TT, Srivareerat M, Alkadhi KA (2011) Chronic psychosocial stress accelerates impairment of long-term memory and late-phase long-term potentiation in an at-risk model of Alzheimer's disease. Hippocampus 21:724-732.

Trejo JL, Carro E, Torres-Aleman I (2001) Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. The Journal of Neuroscience 21:1628-1634.

Tuon T, Valvassori SS, Lopes-Borges J, Luciano T, Trom CB, Silva LA, Quevedo J, Souza CT, Lira FS, Pinho RA (2012) Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. Neuroscience 227:305-312.

Tyler SJ, Dawbarn D, Wilcock GK, Allen SJ (2002) alpha- and beta-secretase: profound changes in Alzheimer's disease. Biochemical and Biophysical Research Communications 299:373-376.

Tyler WJ, Zhang XL, Hartman K, Winterer J, Muller W, Stanton PK, Pozzo-Miller L (2006) BDNF increases release probability and the size of a rapidly recycling vesicle pool within rat hippocampal excitatory synapses. The Journal of Physiology 574:787-803.

Uda M, Ishido M, Kami K, Masuhara M (2006) Effects of chronic treadmill running on neurogenesis in the dentate gyrus of the hippocampus of adult rat. Brain Research 1104:64-72.

Um HS, Kang EB, Leem YH, Cho IH, Yang CH, Chae KR, Hwang DY, Cho JY (2008) Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. International Journal of Molecular Medicine 22:529-539.

Unger JW, Lapham LW, McNeill TH, Eskin TA, Hamill RW (1991) The amygdala in Alzheimer's disease: neuropathology and Alz 50 immunoreactivity. Neurobiology of Aging 12:389-399.

Uysal N, Sisman AR, Dayi A, Aksu I, Cetin F, Gencoglu C, Tas A, Buyuk E (2011) Maternal exercise decreases maternal deprivation induced anxiety of pups and correlates to increased prefrontal cortex BDNF and VEGF. Neuroscience Letters 505:273-278.

Valla J, Schneider L, Niedzielko T, Coon KD, Caselli R, Sabbagh MN, Ahern GL, Baxter L, Alexander G, Walker DG, Reiman EM (2006) Impaired platelet mitochondrial activity in Alzheimer's disease and mild cognitive impairment. Mitochondrion 6:323-330.

van der Borght K, Ferrari F, Klauke K, Roman V, Havekes R, Sgoifo A, van der Zee EA, Meerlo P (2006) Hippocampal cell proliferation across the day: increase by running wheel activity, but no effect of sleep and wakefulness. Behavioural Brain Research 167:36-41.

Van der Borght K, Kobor-Nyakas DE, Klauke K, Eggen BJ, Nyakas C, Van der Zee EA, Meerlo P (2009) Physical exercise leads to rapid adaptations in hippocampal vasculature: temporal dynamics and relationship to cell proliferation and neurogenesis. Hippocampus 19:928-936.

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience2:266-270.

Varela-Nallar L, Inestrosa NC (2013) Wnt signaling in the regulation of adult hippocampal neurogenesis. Frontiers in Cellular Neuroscience 7:100.

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286:735-741.

Vasuta C, Caunt C, James R, Samadi S, Schibuk E, Kannangara T, Titterness AK, Christie BR (2007) Effects of exercise on NMDA receptor subunit contributions to bidirectional synaptic plasticity in the mouse dentate gyrus. Hippocampus 17:1201-1208.

Vaynman S, Ying Z, Gomez-Pinilla F (2003) Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience 122:647-657.

Vaynman S, Ying Z, Gomez-Pinilla F (2004) Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. The European Journal of Neuroscience 20:2580-2590.

Vaynman S, Ying Z, Wu A, Gomez-Pinilla F (2006a) Coupling energy metabolism with a mechanism to support brain-derived neurotrophic factor-mediated synaptic plasticity. Neuroscience 139:1221-1234.

Vaynman SS, Ying Z, Yin D, Gomez-Pinilla F (2006b) Exercise differentially regulates synaptic proteins associated to the function of BDNF. Brain Research 1070:124-130.

Venturelli M, Scarsini R, Schena F (2011) Six-month walking program changes cognitive and ADL performance in patients with Alzheimer. American Journal of Alzheimer's Disease and Other Dementias 26:381-388.

Vianney J-M, Mccullough MJ, Gyorkos AM, Spitsbergen JM (2013) Exercise-dependent regulation of glial cell line-derived neurotrophic factor (GDNF) expression in skeletal muscle and its importance for the neuromuscular system. Frontiers in Biology 8:101-108.

Vitiello MV, Borson S (2001) Sleep disturbances in patients with Alzheimer's disease: epidemiology, pathophysiology and treatment. CNS Drugs 15:777-796.

Vivar C, Potter MC, van Praag H (2013) All about running: synaptic plasticity, growth factors and adult hippocampal neurogenesis. Current Topics in Behavioral Neurosciences 15:189-210.

Vivar C, van Praag H (2013) Functional circuits of new neurons in the dentate gyrus. Frontiers in Neural Circuits 7:15.

Vollert C, Zagaar M, Hovatta I, Taneja M, Vu A, Dao A, Levine A, Alkadhi K, Salim S (2011) Exercise prevents sleep deprivation-associated anxiety-like behavior in rats: potential role of oxidative stress mechanisms. Behavioural Brain Research 224:233-240.

Voss MW, Erickson KI, Prakash RS, Chaddock L, Kim JS, Alves H, Szabo A, Phillips SM, Wojcicki TR, Mailey EL, Olson EA, Gothe N, Vieira-Potter VJ, Martin SA, Pence BD, Cook MD, Woods JA, McAuley E, Kramer AF (2013) Neurobiological markers of exercise-related brain plasticity in older adults. Brain, Behavior, and Immunity 28:90-99.

Wang JT, Medress ZA, Barres BA (2012) Axon degeneration: molecular mechanisms of a self-destruction pathway. The Journal of Cell Biology 196:7-18.

Wang L, Mascher H, Psilander N, Blomstrand E, Sahlin K (2011) Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle. Journal of Applied Physiology 111:1335-1344.

Wang P, Wang WP, Sun Z, Wang HX, Yan L, Fan YH (2008a) Impaired spatial learning related with decreased expression of calcium/calmodulin-dependent protein kinase IIalpha and cAMP-response element binding protein in the pentylenetetrazol-kindled rats. Brain Research 1238:108-117.

Wang X, Su B, Fujioka H, Zhu X (2008b) Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. The American Journal of Pathology 173:470-482.

Wenk GL (2004) Assessment of spatial memory using the radial arm maze and Morris water maze. Current Protocols in Neuroscience / editorial board, Jacqueline N Crawley [et al] Chapter 8:Unit 8 5A.

Wevers A, Burghaus L, Moser N, Witter B, Steinlein OK, Schutz U, Achnitz B, Krempel U, Nowacki S, Pilz K, Stoodt J, Lindstrom J, De Vos RA, Jansen Steur EN, Schroder H (2000) Expression of nicotinic acetylcholine receptors in Alzheimer's disease: postmortem investigations and experimental approaches. Behavioural Brain Research 113:207-215.

Widenfalk J, Olson L, Thoren P (1999) Deprived of habitual running, rats downregulate BDNF and TrkB messages in the brain. Neuroscience Research 34:125-132.

Williams CM, El Mohsen MA, Vauzour D, Rendeiro C, Butler LT, Ellis JA, Whiteman M, Spencer JP (2008) Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. Free Radical Biolology and Medicine 45:295-305.

Wilson JS, Mruthinti S, Buccafusco JJ, Schade RF, Mitchell MB, Harrell DU, Gulati NK, Miller LS (2009) Anti-RAGE and Abeta immunoglobulin levels are related to dementia level and cognitive performance. The Journals of Gerontology Series A, Biological Sciences and Medical Sciences 64:264-271.

Wipfli BM, Rethorst CD, Landers DM (2008) The anxiolytic effects of exercise: a meta-analysis of randomized trials and dose-response analysis. Journal of Sport & Exercise Psychology 30:392-410.

Wisniewski HM, Narang HK, Terry RD (1976) Neurofibrillary tangles of paired helical filaments. Journal of the Neurological Sciences 27:173-181.

Woodruff-Pak DS, Papka M, Romano S, Li YT (1996) Eyeblink classical conditioning in Alzheimer's disease and cerebrovascular dementia. Neurobiology of Aging 17:505-512.

Wu CW, Chang YT, Yu L, Chen HI, Jen CJ, Wu SY, Lo CP, Kuo YM (2008) Exercise enhances the proliferation of neural stem cells and neurite growth and survival of neuronal progenitor cells in dentate gyrus of middle-aged mice. Journal of Applied physiology 105:1585-1594.

Wu L, Rosa-Neto P, Hsiung GY, Sadovnick AD, Masellis M, Black SE, Jia J, Gauthier S (2012) Early-onset familial Alzheimer's disease (EOFAD). The Canadian Journal of Neurological Sciences39:436-445.

Xia Z, Storm DR (2012) Role of signal transduction crosstalk between adenylyl cyclase and MAP kinase in hippocampus-dependent memory. Learning & Memory 19:369-374.

Xiang X, Huang W, Haile CN, Kosten TA (2011) Hippocampal GluR1 associates with behavior in the elevated plus maze and shows sex differences. Behavioural Brain Research 222:326-331.

Yamada K, Fuji K, Nabeshima T, Kameyama T (1990) Neurotoxicity induced by continuous infusion of quinolinic acid into the lateral ventricle in rats. Neuroscience Letters 118:128-131.

Yamamori E, Asai M, Yoshida M, Takano K, Itoi K, Oiso Y, Iwasaki Y (2004) Calcium/calmodulin kinase IV pathway is involved in the transcriptional regulation of the corticotropin-releasing hormone gene promoter in neuronal cells. Journal of Molecular Endocrinology 33:639-649.

Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M, Takao K, Tanda K, Ohira K, Toyama K, Kanzaki K, Fukunaga K, Sudo Y, Ichinose H, Ikeda M, Iwata N, Ozaki N, Suzuki H, Higuchi M, Suhara T, Yuasa S, Miyakawa T (2008) Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. Molecular Brain 1:6.

Yamin G (2009) NMDA receptor-dependent signaling pathways that underlie amyloid beta-protein disruption of LTP in the hippocampus. Journal of Neuroscience Research 87:1729-1736.

Yan L, Li L, Han W, Pan B, Xue X, Mei B (2013) Age-related neuropsychiatric symptoms in presenilins conditional double knockout mice. Brain Research Bulletin.

Yao H, Huang YH, Liu ZW, Wan Q, Ding AS, Zhao B, Fan M, Wang FZ (1998) The different responses to anoxia in cultured CA1 and DG neurons from newborn rats. Acta physiologica Sinica 50:61-66.

Yao W, Zou HJ, Sun D, Ren SQ (2013) Abeta induces acute depression of excitatory glutamatergic synaptic transmission through distinct phosphatase-dependent mechanisms in rat CA1 pyramidal neurons. Brain Research 1515:88-97.

Yassa MA, Stark CE (2011) Pattern separation in the hippocampus. Trends in neurosciences 34:515-525.

Ye X, Tai W, Zhang D (2012) The early events of Alzheimer's disease pathology: from mitochondrial dysfunction to BDNF axonal transport deficits. Neurobiology of Aging 33:1122 e1121-1110.

Yin YY, Liu H, Cong XB, Liu Z, Wang Q, Wang JZ, Zhu LQ (2010) Acetyl-L-carnitine attenuates okadaic acid induced tau hyperphosphorylation and spatial memory impairment in rats. Journal of Alzheimer's Disease 19:735-746.

Ying Z, Roy RR, Edgerton VR, Gomez-Pinilla F (2005) Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. Experimental Neurology 193:411-419.

Young SN (2007) How to increase serotonin in the human brain without drugs. Journal of Psychiatry & Neuroscience 32:394-399.

Yu F, Kolanowski A (2009) Facilitating aerobic exercise training in older adults with Alzheimer's disease. Geriatric Nursing 30:250-259.

Yuede CM, Zimmerman SD, Dong H, Kling MJ, Bero AW, Holtzman DM, Timson BF, Csernansky JG (2009) Effects of voluntary and forced exercise on plaque deposition, hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease. Neurobiology of Disease 35:426-432.

Zagaar M, Alhaider I, Dao A, Levine A, Alkarawi A, Alzubaidy M, Alkadhi K (2012) The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. Neurobiology of Disease 45:1153-1162.

Zagaar M, Dao A, Levine A, Alhaider I, Alkadhi K (2013) Regular exercise prevents sleep deprivation associated impairment of long-term memory and synaptic plasticity in the CA1 area of the hippocampus. Sleep 36:751-761.

Zhao D, Watson JB, Xie CW (2004) Amyloid beta prevents activation of calcium/calmodulin-dependent protein kinase II and AMPA receptor phosphorylation during hippocampal long-term potentiation. Journal of Neurophysiology 92:2853-2858.

Zhao WQ, Ravindranath L, Mohamed AS, Zohar O, Chen GH, Lyketsos CG, Etcheberrigaray R, Alkon DL (2002) MAP kinase signaling cascade dysfunction specific to Alzheimer's disease in fibroblasts. Neurobiology of Disease 11:166-183.

Zhou J, Zhang F, Zhang Y (2000) Corticosterone inhibits generation of long-term potentiation in rat hippocampal slice: involvement of brain-derived neurotrophic factor. Brain Research 885:182-191.

Zlomuzica A, Viggiano D, Degen J, Binder S, Ruocco LA, Sadile AG, Willecke K, Huston JP, Dere E (2012) Behavioral alterations and changes in Ca/calmodulin kinase II levels in the striatum of connexin36 deficient mice. Behavioural Brain Research 226:293-300.

Zoladz JA, Pilc A (2010) The effect of physical activity on the brain derived neurotrophic factor: from animal to human studies. Journal of Physiological Pharmacology 61:533-541.