Bachelor Degree Project

UNIVERSITY OF SKÖVDE

Exercise-induced adult hippocampal neurogenesis and the effect of exercise and adult hippocampal neurogenesis on spatial learning and memory

Bachelor Degree Project in Cognitive Neuroscience
Basic level 22,5 ECTS
Spring term 2018

André Sturesson

Supervisor: Jimmy Andersson
Examiner: Joel Parthemore

Abstract

It was long believed within the scientific community that the adult brain was unable to generate new neurons. In the end of the 1990s the consensus changed and it is since believed that the adult brain can and does generate new neurons after birth, a process referred to as adult neurogenesis. Adult neurogenesis takes place in two places in the adult brain: the subventricular zone (SVZ) in close proximity to the olfactory bulb and the subgranular zone (SGZ) in the hippocampus. The level of adult hippocampal neurogenesis (AHN) can be upregulated and one part of the aim was to examine the effect of voluntary chronic aerobic exercise (VCAE) on AHN. It is clear that voluntary chronic aerobic exercise reliably increases AHN. Still, the function of these new brain cells is under debate. Spatial learning and memory are among the main abilities that have been focused on. The other part of the aim was to examine the effect of VCAE and AHN on spatial learning and memory. The reviewed literature suggests that both AHN and spatial learning and memory increase together from VCAE, although it does not show causation, that an increase of AHN from VCAE causally effects spatial learning and memory. More studies are needed to investigate if a causal relationship exists.

Keywords: adult hippocampal neurogenesis, AHN, exercise, exercise-induced AHN, spatial learning and memory

Table of contents

1. Introduction

When I was in Seventh Grade one of my teachers told me and my classmates that it was very important for us to take care of our brain. I remember him especially saying that each time a person has a night out and consumes alcohol, a thousand brain cells die. I took his opinions as truth and remember being scared but also fascinated. He also claimed that the number of brain cells in the brain was reduced forever, as the brain was incapable of generating new brain cells. Was he right? My teacher's thoughts at the time were in alignment with general opinion within the scientific community regarding the generation of new brain cells (Molina-Navarro & García-Verdugo, 2016). During the 1960s, consensus in the scientific community was that the adult brain could not generate new cells (Molina-Navarro & García-Verdugo, 2016). This line of thought prevailed until the end of the 1990s when the consensus changed. The general opinion from the 1990s onwards is that the adult brain can and does generate neurons (Eriksson et al., 1998).

This ability to generate new brain cells is referred to as adult neurogenesis (Gonçalves, Schafer, & Gage, 2016). Adult neurogenesis takes place in two locations: the subgranular zone (SGZ) in the hippocampus and the subventricular zone (SVZ) close to the olfactory bulb (Voss, Vivar, Kramer, & van Praag, 2013). The ability to generate neurons in these areas is present throughout life in both rodents and humans (Eriksson et al., 1998; van Praag, Shubert, Zhao, & Gage, 2005). Levels of neurogenesis in the SGZ are not set; rather adult hippocampal neurogenesis (AHN) can be up- (van Praag et al., 2005) and downregulated (Kuhn, Dickinson-Anson, & Gage, 1996).

Factors that have been shown to downregulate AHN are aging (Kuhn et al., 1996), stress (Yau et al., 2011), alcohol (Herrera et al., 2003), cocaine (Domínguez-Escribà et al., 2006) and the opiates morphine and heroin (Eisch, Barrot, Schad, Self, & Nestler, 2000). The neurodegenerative diseases Parkinson's disease (Höglinger et al., 2004), Alzheimer's disease (Verret, Jankowsky, Xu, Borchelt, & Rampon, 2007) and Huntington's disease also downregulate AHN (Simpson et al., 2011). All the factors lead to cognitive deficits (Canales, 2016; Herrera et al., 2003; Man Lau, Yau, Po, & So, 2016; Noonan, Choi, Self, & Eisch, 2008; van Praag et al., 2005; Yau et al., 2011). Some authors suggest that the decrease in AHN might contribute to the deficits (Canales, 2016; Herrera et al., 2003; Man Lau et al., 2016; Noonan et al., 2008).

As down regulation of AHN is associated with cognitive deficits, it is of importance to prevent this down regulation and even find ways to increase AHN. Things that have been suggested to upregulate AHN are omega-3 (Kawakita, Hashimoto, & Shido, 2006), antidepressants (Malberg, Eisch, Nestler, & Duman, 2000), curcumin (Dong et al., 2012), and caloric restriction (CR) (Lee, Duan, Long, Ingram, & Mattson, 2000). Curcumin is an active ingredient of the spice turmeric. CR involves limiting total caloric intake compared to baseline by 20-40% without malnutrition: e.g. not having a lack of vitamins and minerals (Murphy & Thuret, 2016). Learning (Gould, Beylin, Tanapat, Reeves, & Shors, 1999), environmental enrichment (Mustroph et al., 2012) and exercise (van Praag, Kempermann, & Gage, 1999) have also been shown to upregulate AHN. Environmental enrichment consists of an environment that provides increased sensory, social or motor stimulation (Mustroph et al., 2012). An example of an enriched environment regarding mice includes toys, treats and a running wheel. There are far more rodent studies of the effects of exercise on AHN compared to the other factors (Hamilton & Rhodes, 2015).

The function of new neurons is under debate (Gonçalves et al., 2016). In rodents it have been suggested that they might play a role in cognitive abilities such as contextual fear conditioning (Wojtowicz, Askew, & Winocur, 2008), pattern separation (Creer, Romberg, Saksida, van Praag, & Bussey, 2010) and spatial learning and memory (van Praag, Christie, Sejnowski, & Gage, 1999). Contextual fear conditioning measures the ability to learn and behave adaptively to aversive stimuli such as shocks. Pattern separation is the ability to recognise small differences between two very similar stimuli: e.g., objects, faces, scenes, experiences or textures. Spatial learning can be described as the ability to process environmental information to navigate the environment and recall locations linked to important stimuli (Floresco, 2015). Spatial learning is believed to involve locating cues in relation to other cues and binding them together to form a map of the surroundings. Spatial learning is highly dependent on the hippocampus, which is known for its function in learning and memory. Spatial memory can be described as the ability to remember where an object is relative to other objects in an environment (Hamilton & Rhodes, 2015). So down regulation of AHN is associated with deficits in cognitive abilities; up regulations seem to be linked to enhanced abilities; exercise has been shown to induce AHN.

Exercise has been shown to prevent cognitive decline related to both age and neurodegenerative disease (Hillman et al., 2008). Exercise has also been shown to increase the size of the hippocampus and improve spatial memory (Erickson et al., 2011). These effects are linked to increased levels of brain derived neurotrophic factor (BDNF). BDNF is an extracellular (outside the cell) signalling protein included in the group neurotrophic factors (Gonçalves et al., 2016). BDNF is believed to mediate neurogenesis (Erickson et al., 2011). Among four identified neurotrophic factors, BDNF is the most studied (Gonçalves et al., 2016). Exercise improves cognitive performance in older adults, including memory and spatial tasks (Colcombe & Kramer, 2003; Smith et al., 2010). Meta-analyses show that exercise reduces depression and anxiety in non-clinical (Rebar et al., 2015) and clinical populations (Rethorst, Wipfli, & Landers, 2009; Stubbs et al., 2017). One of the underlying mechanisms of the exercise-induced benefits could be AHN, as it has been shown to be associated with various benefits in rodent studies (Hamilton & Rhodes, 2015). Studies with human participants are limited as the best methods to study AHN are highly invasive (van Praag, Christie, et al., 1999; Voss et al., 2013). Methods to study AHN in humans are being developed but they still need to be validated (Pereira et al., 2007). The literature provides several more rodent studies compared to human studies (Hamilton & Rhodes, 2015) and hence rodent studies have been focused on in this thesis.

AHN might help to better understand and perhaps aid humans keeping cognitive abilities throughout life. There are several studies that examine the effect of exercise-induced AHN on spatial learning and memory in rodents (Hamilton & Rhodes, 2015). The aim of this thesis is to examine how voluntary, chronic, aerobic exercise (VCAE) affects adult hippocampal neurogenesis (AHN) and in turn, how VCAE and AHN affect spatial learning and memory, focusing on rodent studies.

Types of exercise will be described first. AHN and the brain structures linked to AHN will be described second. Afterwards different factors that increase AHN in rodents will be examined. Exercise-induced AHN will be particularly focused on. The effect of exercise on cognitive abilities of rodents will be described, focusing on spatial learning and memory.

2. Types of exercise

Physical activity is body movement and can be done in myriad ways at different intensities. An example of physical activity is walking to school, another mowing the

lawn. Exercise is body movement that is planned and structured with an end goal. The goal can vary, examples being increasing wellbeing, cardiovascular fitness or building muscle. Examples of exercise are lifting weights at the gym, jogging or dancing.

According to Caspersen, Powell and Christenson (1985) physical activity in humans includes bodily movement conducted by skeletal muscles that results in energy usage. Exercise is a part of physical activity, only it's planned, structured, repetitive and has a goal of improving physical fitness. Exercise in rodent studies is synonymous with physical activity (Voss et al., 2013).

2.1 Chronic and acute exercise

The neurological effects of exercise are both acute and chronic (Hamilton & Rhodes, 2015). Acute effects are those that take place right after the exercise is complete. These wear off and include elevated blood pressure, increased heart rate and increased levels of adrenaline in the blood. After several bouts of exercise per week, during weeks and months, effects can be seen in the brains morphology (Hamilton & Rhodes, 2015). These include more neurons in the DG in rodents (van Praag, Kempermann et al., 1999) together with an enhancement of learning and memory and pattern separation (Voss et al., 2013).

2.2 Aerobic

Aerobic exercise is focused on increasing cardiovascular fitness as well as lung capacity (Seo et al., 2014). Voluntary and involuntary forms exist. The most used voluntary form regarding rodents is wheel running. An example of involuntary exercise is treadmill running. Aerobic exercise is used to study aging, brain function, cognitive abilities, coronary artery disease, endurance capacity, heart function, metabolic syndrome and diabetes (Seo et al., 2014).

2.2.1 *Voluntary exercise*

Most often the rodent has a running wheel in its cage that it can use whenever it wants (Hamilton & Rhodes, 2015). The distance that rodents run is calculated by multiplying the number of revolutions times the circumference of the wheel during a 24-hour period. The distance that different strains run voluntarily varies, from approximately three kilometers to ten kilometers per day (Clark et al., 2011). The running patterns include short bouts of fast running with short breaks (Hamilton & Rhodes, 2015). Wheel running is suggested to be more similar to riding a bike than running when making a human analogue. Advantages of wheel running are that it is simple to use, avoids stressing the animals, reliable in that the distance the rodent

travels is similar from day to day. Disadvantages include no control over the distance the animal runs, difficulty examining high-intensity exercise, as well as comparability to humans (Hamilton & Rhodes, 2015). Humans most often exercise in 20-40 min bouts a couple of times per week.

2.2.2 *Forced exercise*

Treadmills are often used in forced exercise studies with rodents (Hamilton & Rhodes, 2015). Treadmill running has some limitations; it is time-consuming because of the necessity to often train the rodents to cooperate. Rodents are intermittent runners and running continuously is not natural for them. Treadmill running is believed to induce psychological stress, which can be a confounding variable (Seo et al., 2014). Often the animals need some type of motivating stimuli to run such as an electric grid or a foam pad (Hamilton & Rhodes, 2015). The challenge of getting the rodents to cooperate often results in low speed of the treadmill as higher speeds produce more cooperation problems. Forced running also has advantages. One being the control of the amount of exercise the rodents perform (Seo et al., 2014). Forced exercise can be better matched to how humans exercise (Hamilton & Rhodes, 2015).

2.3 Anaerobic

Anaerobic exercise, also referred to as resistance training, aims to increase muscle strength (Seo et al., 2014). Rodents do not normally lift weights, making resistance training problematic for them (Hamilton & Rhodes, 2015). One method has been to restrain rats in an apparatus in which they perform squats, believed to mimic human squats (Seo et al., 2014). A major disadvantage is the frequent use of noxious stimuli to make the rodent do the squats. Another example is ladder climbing (Seo et al., 2014). In ladder climbing rats are trained to climb a ladder with weights attached to their tail. This exercise is progressive: weights are increased step by step. Ladder climbing is less stressful for the animals once they have become familiar with the exercise and is often referred to as *voluntary after familiarisation* (Seo et al., 2014).

3. Adult neurogenesis

3.1 History

The general opinion during the 1960s was that the adult brain was not capable of creating new neurons after birth (Molina-Navarro & García-Verdugo, 2016). One researcher studying cell division questioned this; Joseph Altman, a researcher originally from Hungary investigated cell *proliferation* in the brains of cats and mice (Altman & Das, 1965). Proliferation can be referred to as a phase during neurogenesis

when cells multiply. Jospeh Altman became the first to propose that new brain cells were created in the dentate gyrus of the adult mammal brain. The idea was controversial and ignored during two decades (Molina-Navarro & García-Verdugo, 2016; Lois & Kelsch, 2014). Twenty years later the phenomenon was studied again. Kaplan was examining micro-environments in the brain, publishing articles on adult neurogenesis in the hippocampus (Molina-Navarro & García-Verdugo, 2016). Simultaneously, Fernando Nottebohm was the first to study neurogenesis in birds. His research suggested that AHN was related to learning, paving the way for studies regarding the function of neurogenesis (Molina-Navarro & García-Verdugo, 2016). In the 1990s, various studies was conducted and the field grew (Molina-Navarro & García-Verdugo, 2016). One study suggested a link between AHN and stress, stating that adrenal hormones supress the birth of neurons in the adult rat dentate gyrus (Gould, Cameron, Daniels, Woolley, & McEwen, 1992). Another study addressed the birth, movement and development of newborn cells in birds (Alvarez-Buylla, 1990). In the end of the 1990s AHN was generally accepted within the scientific community and had been proven to occur in several species, examples being mice (van Praag, Kempermann, et al., 1999) but also primates such as marmoset monkeys (Gould, Tanapat, McEwen, Flügge, & Fuchs, 1998) and macaque monkeys (Kornack & Rakic, 1999). Eriksson et al. (1998) were the first to show AHN in humans, using bromodeoxyuridine (BrdU). BrdU is used to label and distinguish proliferating cells. New neurons were demonstrated to be created in the dentate gyrus of the adult human. The results indicate the ability for the hippocampus to generate neurons throughout the entire life of a human being. In 2010 the field of neurogenesis was one of the fastest growing fields in neuroscience (Lucassen et al., 2010). The last ten years have led to substantial progress in understanding adult neurogenesis and its function (Gonçalves et al., 2016).

3.2 Adult neurogenesis (AHN)

Neurogenesis can be defined as a process that results in the generation of neurons from neural stem cells (NSCs) or progenitor cells (Jin, 2016). Adult neurogenesis is the continuance of embryonic neurogenesis (Molina-Navarro & García-Verdugo, 2016).

Neuroepithelial (NEP) cells generate neurons in the embryonic neural tube during the early stages of mammalian brain development (Jin, 2016); radial glial cells (RGCs) generates both neurons and glial cells during embryonic development

(Hartfuss, Galli, Heins, & Götz, 2001). Generation of neurons before birth is more pervasive compared to after (Jin, 2016).

Adult neurogenesis is the continuance of embryonic neurogenesis, taking place in two neurogenic niches (micro-environments) in the adult mammalian brain (Gonçalves et al., 2016; Lledo, Alonso, & Grubb, 2006): the sub ventricular zone (SVZ) near the olfactory bulb (Lledo et al., 2006) and the sub granular zone (SGZ) of the hippocampal dentate gurys (DG) (Gonçalves et al., 2016). Estimations have suggested that the young adult rodent generates 250,000 new granule cells each month in the dentate gyrus, which amounts to 9,000 per day. This is suggested to be 6% of the total amount of granule cells in the DG in the adult hippocampus. The generation of these neurons supports the idea that neurogenesis is linked to hippocampal function (Cameron & McKay, 2001). Estimates have also been done concerning the amount of new neurons in the human hippocampus per day. Spalding et al. (2013) proposed that 700 neurons are generated each day. They estimated that 1.75% of the neurons within the human hippocampus are renewed each year.

Neurogenesis is a form of brain plasticity (Gonçalves et al., 2016). Brain plasticity can be defined as the brain's ability to change its structure and function (Kolb & Whishaw, 1998). Brain plasticity can be categorised as experienceexpectant, experienceindependent and experiencedependent (Kolb & Gibb, 2014). Experienceexpectant plasticity happens to great extent during development. Experience helps shape development of the brain. Experienceindependent plasticity is linked to a surplus of neurons during development that is gradually decreased. Neurons that are needed survive; those that are not are much more likely to degenerate. Experiencedependent plasticity is the change of neurons due to experience: e.g., physical exercise that leads to new cells in the hippocampus (van Praag, Christie, et al., 1999).

3.2.1 Hippocampus

The hippocampus is a structure in the brain most known for its function in learning and memory. One of the most famous amnesic patients, H.M, had a brain operation in 1953 because of epilepsy (Augustinack et al., 2014). The surgery led to the removal of substantial parts of the hippocampus resulting in anterograde amnesia, a disorder linked to memory. The disorder results in memory impairments related to creating new long-term memories that can be declared. The memory impairments were evident in everyday situations for H.M (Gazzaniga, Ivry, & Mangun, 2013).

The ability to learn and memorize aids in our adaption to the environment: e.g., by remembering what to avoid and what to seek out (Gazzaniga et al., 2013). Learning is the acquisition of new information, and memory is the outcome. The acquired information can last for different amounts of time: e.g. briefly or for a lifetime.

3.2.2 Dentate gyrus (DG) & the subgranual zone (SGZ)

The dentate gyrus is a major input region to the hippocampus, believed to play an essential role in learning, episodic memory and spatial navigation (Gonçalves et al., 2016). The DG is believed to be involved in discriminating between similar though not identical experiences, an ability known as *pattern separation* (O'reilly & McClelland, 1994). The function of pattern separation has been supported by e.g. hippocampal lesion studies (Gonçalves et al., 2016). The DG seems to be important regarding fear conditioning and freezing behaviour. An example of adaptive freezing behaviour in rodents is becoming still due to a stimuli. Fear conditioning is when a neutral stimulus becomes associated with a noxious stimulus. By labelling hippocampal dentate gyrus neurons during fear condition and then activating them at a different time the mice show increased freezing (Liu et al., 2012). The DG also seems to be involved in memory retrieval (Gonçalves et al., 2016).

The DG looks like a 'V' or a 'U' and is created by three layers: the molecular layer, granule cell layer and hilus (Molina-Navarro & García-Verdugo, 2016). The subgranular zone (SGZ), where neurogenesis takes place (Gonçalves et al., 2016), is a layer of granule cells between the granule cell layer and hilus. The transition between these two layers, the granule cell layer and the hilus, are sharp within rodents. The same area for humans is a serrated border (Molina-Navarro & García-Verdugo, 2016). The micro-environment of the SGZ aids stem cells in their proliferation and differentiation into mature neurons (Gonçalves et al., 2016). Differentiation can be referred to as the process by which the cell develops into its destined cell.

3.2.3 Cell process of AHN

A mature granule cell in the SGZ goes though several stages to become a mature neuron (Gonçalves et al., 2016). These include proliferation, differentiation, maturation and integration. Type 1 radial glia-like cells (RGLs), also referred to as neural stem cells, create intermediate progenitor cells, also referred to as type 2 cells, that proliferate, meaning they divide and increase. Type 2 cells in turn become neuroblasts, type three cells, which differentiate into immature neurons. Finally, the

new neuron matures and is integrated with the other neurons in the SGZ. The SGZ consists of cells within all these stages: stem cells, intermediate progenitors, immature neurons as well as mature granule cells (Gonçalves et al., 2016; Molina-Navarro & García-Verdugo, 2016). When measuring AHN, it is most common to measure the quantity of dividing cells (Gonçalves et al., 2016). This is done by labelling the proliferating cells with BrdU, making them distinguishable. Other measures exist, such as survival of newly generated neurons and the quantity of immature neurons (Gonçalves et al., 2016).

3.2.4 The function of newly generated DG neurons

Understanding of the function of adult newborn neurons has increased rapidly the last decade; even so, no consensus regarding the function of new adult neurons exists (Gonçalves et al., 2016). More experiential data is required.

The improved understanding of AHN and DG have happened in parallel (Gonçalves et al., 2016). Adult-born neurons are not likely to have an effect on behaviour before they integrate into the DG network. These new neurons are believed to be more plastic and have an increased excitability, meaning they more easily fire action potentials, during the forth and sixth week post-mitosis. The increased plasticity and excitability is transient; new neurons eventually mature and receive properties similar to other adult granule neurons (Gonçalves et al., 2016).

Different theories of the function of AHN exists, including encoding temporal information into memories (Aimone, Wiles, & Gage, 2006), pattern separation (Aimone, Wiles, & Gage, 2009) and spatial learning and memory (van Praag, Christie, et al., 1999).

3.3 Increase of AHN

3.3.1 Omega-3

Omega-3 polyunsaturated fatty acids (PUFAs) affect cognitive performance and development of the brain (Innis, 2007). According to Innis docosahexaenoic acid (DHA), one of the PUFAs, plays a role regarding neurogenesis, neurotransmission and protection against oxidative stress. Neurotransmission involves neurotransmitters being sent from one neuron to another within the synaptic cleft and oxidative stress involves free radicals (an uncharged molecule with an unpaired valence electron) that damage cells.

Kawakita et al. (2006) showed enhancement of AHN from omega-3 intake: 18-month-old rats were fed DHA, 700 mg/kg during seven weeks. The increased

intake resulted in significantly increased AHN in the dentate gyrus. The study suggested an increase of proliferation, differentiation and maturation of dentate granule cells. Differentiation is when the cell develops into its destined cell.

He, Qu, Cui, Wang & Kang (2009) conducted a study regarding omega-3 and AHN in mice modified to have increased levels of DHA in the brain, which was shown to significantly enhance neurogenesis in their hippocampus. Changes were seen regarding increased proliferation as well as increased density of dendritic spines. The mice performed better in a spatial learning task compared to control mice.

Other studies suggest an effect from omega-3 concerning brain function. Gamoh, Hashimoto, Hossain and Masumura (2001) divided rats deprived of fish-oil for three generations randomly into two groups. One group were given 300 mg/kg of DHA per day and the control group received no DHA. After five weeks the rats were tested regarding spatial memory. The rats fed with DHA were significantly better concerning spatial memory.

3.3.2 Antidepressants

Malberg et al. (2000) showed that chronic use of antidepressants increases levels of proliferation in the hippocampus of adult rats. The study used different kinds of antidepressants: selective-serotonin reuptake inhibitors (fluxetine), norepinephrine-selective reuptake inhibitors (reboxetine), as well as monoamine oxidase inhibitors (tranlylcypromine). All increased the levels of AHN in the adult rat's hippocampus. Chronic but not acute treatment with antidepressants led to an increase in proliferation, suggesting that it takes time before the effects on AHN from antidepressants can be recognised. The authors suggest that the findings might implicate a mechanism of how antidepressants overcome stress-induced atrophy and loss of hippocampal neurons.

Other studies have showed that antidepressants have an effect on AHN. Keilhoff, Bernstein, Becker, Grecksch and Wolf (2004) showed that ketamine, a fast acting antidepressant, enhances neurogenesis in the dentate gyrus (DG). Rats were injected with ketamine during five consecutive days, resulting in an enhancement of AHN. Two control groups were used: one received saline solution and one no treatment at all. These two groups did not show the increase of proliferating cells in the DG.

3.3.3 Curcumin

Dong et al. (2012) examined memory performance and hippocampal cell proliferation in older rats linked to curcumin. The rats were given curcumin diets during six and twelve weeks. The study resulted in an increase of proliferation in the dentate gyrus compared to controls. The study also showed a positive effect of curcumin on both non-spatial and spatial memory although the increase in spatial memory was only significant in the mice who had received curcumin during twelve weeks and not six weeks. Conboy et al. (2009) gave curcumin to adult and older rats during eight days. Both the adult and older rats significantly improved their performance in the *Morris water maze* indicating improvement of spatial learning and memory. The Morris water maze is a test that measures spatial learning and memory by having rodents learn where a hidden platform is using environmental cues (Vorhees & Williams, 2006). A pool including a hidden platform that the rodent can stand on is filled with water. The rodents are inserted into the pool; the aim for the rodent is to find and remember the hidden platform. The rodents do several trials per study. Each trial lasts until the rodent has either found the platform or the time is up (between one and two minutes). If the rodent fails to find the platform before the time is up, it is guided to or placed on the platform (Vorhees & Williams, 2006).

3.3.4 Caloric restriction

Caloric restriction (CR) is believed to be a stress response that has evolved to increase chances of survival during adversity (Sinclair, 2005). CR has been shown to increase the lifespan of species such as worms, flies, yeast and rodents (Sinclair, 2005). Lee et al. (2000) showed that caloric restriction has an effect on AHN in rats. Their study showed that three months of caloric restriction of 30% starting when the rats were three months old increased AHN due to a decrease in cell death of newly produced neurons, and not cell proliferation. The increase in AHN was linked to elevated levels of BDNF in the hippocampus. Lee, Seroogy and Mattson (2002) did a study with similar results showing that CR increased cell survival of new neurons in the DG and increased BDNF. Adams et al. (2008) demonstrated that lifelong CR could prevent age-related declines of hippocampal-dependent tasks such as spatial learning in rats.

3.3.5 Learning

Studies suggest that learning increases the rate of neurogenesis (Gould et al., 1999). Gould et al. used both hippocampus-dependent and hippocampus-independent

learning tasks to look at their effect on AHN. The hippocampus-dependent task resulted in an increase of new neurons in the DG. The hippocampus-independent tasks did not effect the number of new neurons in the DG. Interestingly, the number of cells did not increase because of the learning task; instead neurons generated before the training had increased survival. Kempermann and Gage (2002) established a link between learning measured by the Morris water maze and neurogenesis. Different strains of mice having different levels of baseline AHN were used to find a correlation between the numbers of new neurons created in the DG as well as the learning curve in the Morris water maze. The study showed a correlation between the learning curve in the Morris water maze and the number of newly generated neurons per day in the DG. These studies indicate that AHN is increased by learning and learning is increased by AHN.

3.3.6 Environmental enrichment

Rodents living in enriched environments have increased AHN as well as increased performance on the Morris water maze, indicating an increase in spatial learning (Mustroph et al., 2012; Kobilo et al., 2011). Mustroph et al. (2012) divided mice into four groups: a standard control group (group A) with an empty cage, no running wheel, toys or treats. Group B had an enriched environment, toys and treats, although without the running wheel. Group C had an empty cage with only a running wheel. Group D had an enriched environment with toys and treats as well as a running wheel. There were no difference in increased AHN between group C (running wheel only) and D (running wheel + other aspects of an enriched environment). The only group that showed an increase in spatial learning compared to the control group was the running-only group (group C). The environmental-enrichment-only group, group B, did not show an increase in AHN and no increase in spatial learning compared to the control group. Kobilo et al. (2011) had similar results showing that an increase of AHN was only evident if the mice had a running wheel, independent of other aspects of the enriched environment. Both studies indicate that exercise is the most important factor of an enriched environment relative to AHN enhancement. These two studies and a vast number more have shown that exercise is a reliable way to increase AHN (Gonçalves et al., 2016; Kronenberg et al., 2006; van Praag, Kempermann, et al., 1999; van Praag et al., 2005).

4. Exercise-induced AHN

4.1 Exercise-induced AHN

Van Praag, Kempermann et al. (1999) showed that voluntary aerobic exercise increased cell proliferation as well as survival of new dentate granule cells in three-month-old mice. The mice had free access to a running wheel for 42 days. Van Praag et al. (2005) studied whether increased AHN due to aerobic exercise was evident in older mice that had been sedentary until 19 months of age. These mice had access to a running wheel for 45 days and ran a mean distance of 3.9 km per day. The study revealed that neurogenesis due to exercise increase in sedentary old mice. Age-related decline in neurogenesis was partially reversed by voluntary exercise. Studies exist of increased AHN from voluntary wheel running when mice have been living in a group setting (Kobilo et al., 2011) as well as alone (Marlatt, Potter, Lucassen, & van Praag, 2012). The increase has been seen in studies using only female mice (Marlatt et al., 2012; van Praag, Kempermann, et al., 1999) and only male mice (Kronenberg et al., 2006; van Praag et al., 2005). One study examined voluntary resistance running: running on a running wheel with weight resistance (Lee et al., 2013). The resistance makes the workout more exhausting. The workout level of the rats was progressively increased during a four-week period starting with minimal resistance the first week, which was then progressively increased up to 35% of the rat's body weight. Voluntary resistance running increased AHN similarly to voluntary wheel running, with higher work levels and shorter distances. Voluntary resistance running is not believed to induce stress in the rats.

Nokia et al. (2016) looked at the effects of high-intensity interval training (HIT) on AHN of adult rats. HIT can be explained as short bouts of intense exercise followed by recovery periods. A bout of HIT exercise consisted of 20 minutes. The study concluded that AHN was elevated moderately although non-significantly.

The authors used genetically different rats to see if there was an effect of genes linked to the effect of increased AHN. The study separated rats into high and low responders to exercise (HRT and LRT). Nokia et al. found that HRT rats had increased exercise-induced AHN compared to LRT rats.

Clark et al. (2011) showed that exercised-induced AHN varied across strains of voluntary running mice. The mean distance ran each day varied from approximately three kilometers to ten kilometers, depending on strain. The study found that the mean distance ran within each strain correlated with the levels of

neurogenesis indicating a dose-response relationship between distance travelled and AHN increase among genetically similar mice. Longer distance travelled within a strain resulted in more increase in AHN. The study also showed differences between strains regarding how far the mice ran and how much AHN increase they had. The magnitude of exercise-induced AHN was most evident in one strain named AKR/J which did not run the furthest distance. AKR/J ran approximately 4.6 km per day and had the highest increase of AHN compared to baseline. The most commonly used mice in exercise-induced AHN studies, C57BL6/J, ran approximately 4.4 km per day and had the lowest total increase of AHN compared to baseline: 1.6-fold. C57BL6/J had the highest baseline AHN of all the strains. Different strains had different numbers of surviving neurons after exercising. B6129SF1/J and AKR/J mice had the highest number of surviving granule cells and 129S1/SvlmJ mice had the lowest. C57BL6/J came on tenth place of twelve in regards of surviving neurons. AKR/J had the highest amount of surviving cells implying that genes, again, is a factor. Finally, all twelve strains had increased AHN.

Forced exercise in this case with the use of treadmills has also been shown to increase AHN (Kim et al., 2014; Li et al., 2013).

4.1.1 Underlying mechanisms of exercise-induced AHN

Several growth factors have been shown to regulate adult hippocampal neurogenesis (Gonçalves et al., 2016) and to be upregulated by exercise (Voss et al., 2013). Growth factors consist of a large group of extracellular proteins that control both maintenance and growth of cells (Gonçalves et al., 2016). Growth factors that are believed to effect AHN include brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) (Voss et al., 2013).

4.1.1.1 *BDNF*

The maturation of neurons is accelerated by BDNF, linked to dendritic growth, cell differentiation and complexity (Gonçalves et al., 2016; Taliaz, Stall, Dar, & Zangen, 2010). After a bout of exercise, BDNF is increased in the hippocampus, suggested to stimulate AHN (Cotman & berchtold, 2002). Taliaz et al. (2010) did a study where BDNF was reduced significantly by knocking down BDNF expression. Decreased BDNF expression resulted in reduced neurogenesis.

4.1.1.2 *IGF-1*

IGF-1 is a growth factor increased by exercise (Trejo, Carro, & Torres-Aleman, 2001). Studies indicate that IGF-1 influences both proliferation and differentiation of

neurons and glia cells as well as contributing to neuronal maturation (Gonçalves et al., 2016; Nieto-Estévez, Defterali, & Vicario-Abejón, 2016). Exercise-induced AHN is removed when IGF-1 is blocked in rats (Trejo et al., 2001).

4.1.1.3 *VEGF*

Vascular Endothelial Growth Factor (VEGF) is a neurotrophin belonging to the class growth factors (Fabel et al., 2003). VEGF is increased by aerobic training and stimulates both creation of new blood vessels (angiogenesis) and neurogenesis. Fabel et al. (2003) examined the effects on AHN when VEGF induced by exercise was blocked. The exercise-induced increase of AHN did not occur.

5. Effect of exercise and AHN on spatial learning and memory

5.1 The effect of chronic exercise on cognition

The question does not seem to be whether exercise enhances AHN but rather what the function is of these new cells. Increased AHN has been examined in relation to abilities such as contextual fear conditioning, motor performance (Clark et al., 2008), pattern separation, spatial learning and memory.

Wojtowicz et al. (2008) studied motor function by reducing AHN in one group of adult rats by radiation and increased in one group of adult rats by exercise. This study indicated that reduced neurogenesis severely degraded contextual fear conditioning. A reduction in neurogenesis produced a reduction in freezing. By contrast, an increase in neurogenesis resulted in an increase in freezing behaviour.

AHN has been suggested to increase behavioural performance on the *rotarod* test for rodents (Clark et al., 2008). The rotarod test is a test of motor performance including balance and coordination comprised of the rodents being placed on a spinning dowel. The rodent is timed to measure how long it can stay on the dowel. Exercising rats had a fourfold increase of AHN compared to sedentary rats and did better on the rotarod test, indicating better motor performance.

Creer et al. (2010) investigated whether AHN increases pattern separation in mice. Two different ages of mice were used. Both three and twenty-two-month-old mice were given either an empty cage or a cage with a running wheel, four groups in total. The mice were then tested to see if there was a difference in pattern separation between exercising and sedentary mice. The three-month-old mice with running wheels had an increase of AHN closely correlated to improved performance on the spatial pattern separation task, compared to the sedentary mice of the same age. The

older mice did not show improvement on the pattern separation task regardless if they had a running wheel or not.

Sahay et al. (2011) showed that genetically increasing AHN in mice resulted in improved pattern separation. Inducing enhanced AHN genetically combined with an exercise intervention robustly increased exploratory behaviour. Increasing AHN genetically without the exercise did not.

5.1.1 Spatial learning and memory

Van Praag, Christie et al. (1999) conducted an experiment where three-month-old mice were divided into two groups: runners equipped with a running wheel and sedentary controls living in an empty cage. The running mice ran a mean of 4.78 km per day. The mice living in their cages between two to four months were tested on the water maze between days 30 and 36 with four trials per day, or between days 43 and 49 with two trials per day. A trial is a round in the Morris water maze to find the hidden platform. The running mice had an increase of AHN measured as more proliferating cells compared to controls. They also performed significantly better in the Morris water maze between days 43 and 49 with two trials per day. The authors conclude that it was possible that the increase in adult hippocampal neurogenesis of runners contributed to the better performance on the water maze task. The runners had an increase of dental gyrus long-term potentiation: i.e., strengthening of synapses induced by previous patterns of activation.

Gibbons et al. (2014) equipped mice who were 18 months old with a running wheel and measured proliferation. Spatial learning and memory were examined using the Morris water maze after 28 days of running. These “older” runners had increase in proliferation as well as an enhanced performance on the Morris water maze compared to controls. Van Praag et al. (2005) came to similar conclusions regarding older mice. Mice that had been sedentary until 19 months of age were better in the Morris water maze compared to age matched controls after one month of voluntary exercise. The age-related decline in neurogenesis of the older mice was reversed. Older mice had increased AHN, but still had less AHN (50%) than sedentary young mice who did not exercise.

Marlatt et al. (2012) examined the effects of long-term running on neurogenesis and spatial learning and memory in middle-aged mice. At nine months, mice were randomly assigned to a voluntary running group or a control group. The runners ran a mean of four kilometers per day. Both groups were tested on the Morris

water maze at months ten and fifteen when the running mice had been exercising for one and six months, respectively. There was no difference regarding performance on the Morris water maze at ten months. At fifteen months the runners performed better on the Morris water maze, indicating enhanced spatial learning and memory. After 17 months, the runners had an increase in AHN and BDNF compared to controls. These data suggest that long-term running can be beneficial in protecting against age-related memory decline. The study suggests that the new neurons generated at nine months survive until seventeen months, indicating their long-term survival.

If exercise increases AHN and spatial learning and memory, stress has the opposite effect (Yau et al., 2011). Yau et al. studied the effect of stress and exercise on AHN in rats. Exercising rats were given different doses of corticosterone, a hormone related to stress, either no dose, a low dose (30mg/kg), a moderate dose (40 mg/kg) or a high dose (50 mg/kg) during 14 days. Four non-running control groups matched the runners regarding the coricosterone dose. Proliferation and BDNF were measured and the Morris water maze used to measure spatial learning and memory. The corticosterone resulted in impaired spatial learning and decreased cell proliferation and BDNF in the control rats. Running was shown to counteract the effects of the corticosterone of rats treated with a low or moderate dose of corticosterone.

It is common that strokes induce spatial cognitive impairments (Luo et al., 2007). Luo et al. examined whether voluntary and forced exercise enhanced AHN and reversed deficits in spatial memory after a stroke. The authors induced focal cerebral ischemia to mice: a stroke that leads to cell death in a particular area due to a blood clot. Then mice ran either voluntarily in a running wheel or were forced to swim. Mice who ran voluntarily had enhanced AHN as well as a reversal of the stroke-induced spatial memory impairment. The effects were not seen in forced swimmers. The study showed a correlation between the total number of new-born neurons that survived in the DG and the ability to locate the platform in the water maze.

Merritt & Rhodes (2015) examined the effects from voluntary running on AHN and spatial learning and memory among different strains of mice. Five strains were randomly assigned to a voluntary running group or a sedentary control group. The mice ran or were sedentary for 30 days, then tested on an enhanced version of the water maze on days 31 to 39. On day 42 the mice were anesthetized and their brains removed and analysed. The ‘plus’ water maze is a version of the Morris water maze

adapted to fit multiple strains. The biggest difference between the Morris water maze and the 'plus' water maze is how it is constructed. The plus water maze contains less open space and four different lanes. One lane contains the hidden platform. The running group independent of strain had an increase in the number of new neurons compared to sedentary mice. They also performed better on the water maze compared to controls. The study mentioned a problem about the analysis of the link between levels of exercise-induced AHN and performance on the water maze, there being more than one way to represent an increase of AHN: either as the total amount of new neurons or as a proportional increase compared to the sedentary baseline. If AHN increase is seen as a proportional increase then the data lead to a different suggestion. The strain D2 had the highest increase of neurogenesis compared to baseline, the strain B6 the smallest. Of the five strains, D2's performance on the water maze was the least enhanced by running. This suggest a dissociation between performance on the water maze and increase of AHN levels from running.

Clark et al. (2008) investigated what effect exercise had on spatial learning and memory if AHN was removed. Mice were assigned as either voluntary runners with or without AHN reduction or sedentary mice with or without AHN reduction. Reduction in AHN was induced by focal gamma irritation, a form of radiation that downregulates AHN. Runners without the reduction showed a fourfold increase of neurogenesis and had increased performance on the Morris water maze compared to controls. Runners with the neurogenesis reduction did not show the enhancement on the Morris water maze.

The studies indicate a link between exercise-induced AHN, at least when counted as the total amount of newly generated neurons, and spatial learning and memory. Studies also exist where such enhancements in spatial learning and memory do not take place. Rhodes et al. (2003) investigated mice bred for running (S mice) and control mice (C mice) not bred for running. These mice were randomly assigned to a voluntary running group or control group. The running group of C mice showed increased proliferation and BDNF levels as well as improved spatial learning and memory. There was a strong correlation between running distance and improved learning. In S runners, AHN increased to a high level then halted. BDNF levels also increased. No improvement regarding spatial learning and memory was evident. The authors speculate that the hyperactivity evident in the S mice could have interfered

with the ability to learn. This study was the first not to show enhancement of spatial learning and memory with exercise-induced AHN.

In the previously mentioned study by Luo et al. (2007), the forced exercisers did not show an increase in AHN or enhanced spatial learning and memory. Ang, Dawe, Wong, Moochhala and NG (2006) conducted a similar study where they examined the effects of forced exercise on spatial learning and memory, although without inducing a stroke. They showed that twelve weeks of forced treadmill running did improve spatial learning and memory in rats.

6. Discussion

The aim of this thesis is to examine the effect of voluntary, chronic aerobic exercise (VCAE) on adult hippocampal neurogenesis (AHN), and in turn how VCAE and AHN effect spatial learning and memory, focusing on rodent studies. More knowledge about neurogenesis might help humans sustain and even improve cognitive abilities throughout life. What if AHN can help to reverse cognitive decline, like dementia? If future studies conclude that AHN is beneficial for humans then it is of importance to find a reliable way to enhance AHN which in turn makes reviewing exercise important as exercise has been studied extensively as an intervention to enhance AHN.

VCAE has reliably been shown to increase AHN in rodents including young mice, old mice, female mice, male mice, mice housed individually and mice housed in a group setting. There is little doubt whether this form of exercise increases AHN. Still, it could be argued that rodents living in an empty cage are environmentally deprived and that voluntary running is an enhancement that effects AHN independently of the exercise component, though this seems rather unlikely as forced exercise has also been shown to increase AHN.

The interest in how various types of exercise influence AHN has increased in recent years. In one study high-intensity interval training (HIT) was used (Nokia et al., 2016). This study showed that HIT elevated AHN, although non-significantly. It is too early to say if HIT is an effective way to enhance AHN, as the new method could have interfered with willingness to comply. Another example is voluntary resistance running (Lee et al., 2013). Lee's study showed that resistance running, with its higher workout level and shorter bouts of exercise compared to voluntary wheel running, induces a similar increase of AHN as VCAE. Some forms of exercise may be more beneficial than others in increasing the generation of new neurons, which could

contribute to enhanced brain function. Humans tend to like different forms of exercise; if it is shown that all forms of exercise increase AHN to the same degree, then that would be beneficial as individuals could conduct the form of exercise that they are inclined to. On the other hand, if only some forms of exercise enhance AHN, then it is of importance that individuals receive the right information.

Different strains of rodents have different baseline levels of AHN. Strains also differ in their AHN enhancement from exercise. AKR/J ran a similar distance as C57BL6/J, although AKR/J had a fourfold increase of AHN compared to baseline and C57BL6/J a 1.6-fold increase. The total amount of new brain cells induced from exercise also differed. AKR/J had vastly more new brain cells than C57BL6/J. Nokia et al. (2016) showed that genes are important to exercise-induced AHN by showing that high- and low responders to exercise had different amounts of exercise-induced AHN. High responders had more AHN enhancement from exercise. Future research should try to map the genes that modulate the relationship between exercise and AHN in rodents and humans, especially if more evidence is put forward that shows benefits of having higher levels of AHN. If specific genes can be found that influence both baseline levels of AHN and exercise-induced enhancements of AHN, then those could be used to influence AHN enhancement in the future. It could very well be that some human phenotypes benefit more from exercise compared to others. It could be that some humans have higher baseline levels of AHN and hence perhaps do not need to exercise as much. It could also be that some individuals get significant effects from exercise and some do not. This is especially important as rodents have been shown to have increased AHN in certain circumstances but not increased cognitive abilities: a relationship that might occur in humans as well. Getting humans to start exercising is a difficult task. If resources are to be assigned aiming at getting more people to exercise then it is important that the exercise has effect. Exercise is effective against depression and anxiety. It is also a way to increase executive function. Trying to get more people to exercise is important even if AHN is not the benefactor. This holds true for adolescents, adults and the older population. The question then is how to get more people to exercise. As an exercise routine is something that can be hard to accomplish, I believe in having exercise inserted into the structure of society, into our schools. Having children and adolescents exercise at least twice a week in school, testing different types of physical activity, would be beneficial as children would get accustomed to exercise. This would also aid children in figuring out what they like.

Information about the benefits of exercise should be added to gym class to aid children in understanding why it is beneficial. Many adolescents today have clinical levels of depression and anxiety. Starting early with exercise and explaining the effects of exercise would provide a potent tool to use to counteract depression and anxiety. It is important to counteract the picture of exercise as a tool to effect only looks; instead exercise needs to be linked to the health benefits it provides. Physical activity is a natural medicine evolved during millions of years.

Age has been shown to effect exercise-induced AHN (van Praag et al., 2005). Older mice have lower levels of baseline AHN. Exercise does increase AHN levels in older mice although to a smaller degree than younger mice. Still, older mice do receive an exercise-induced enhancement of AHN, which partially reverses this age-related decline in AHN. It could be that age-related decline in AHN is reversible in humans, which in turn could aid older adults in keeping their cognitive functions, if they participate in chronic aerobic exercise. This is especially important as neurodegenerative diseases have been shown to decrease AHN. Aging affects the brain negatively probably as more things can go wrong but also probably since the brain does not get what it needs, one thing being physical activity. If the older generation would exercise more, that would probably enhance AHN, which could help our older population to preserve their cognitive abilities. What if we could help our older generation in general and our older generation with neurodegenerative diseases to remember individuals important to them: partners, children, grand children and friends? What would that mean for the lives of the affected individuals?

In this thesis the underlying mechanisms that contribute to exercise-induced AHN were examined, mostly focusing on growth factors. Brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) are growth factors that are proposed to enhance exercise-induced AHN (Voss et al., 2013). They have all been shown to enhance AHN and to be upregulated by exercise. Studies have shown that blocking and reducing the effects of these growth factors impairs AHN. A study by Erickson et al. (2011) showed that exercise in older human adults increased the size of their hippocampus with an increase of BDNF, providing a possible link between human and rodent studies regarding BDNF and AHN. This is an important possible link as the brains of humans are hard to study because of the methods of AHN being highly invasive. New methods to study AHN in humans are being developed although they still need to be validated. Looking at

biomarkers such as BDNF, IGF-1 and VEGF can provide support for AHN in humans. Rodent studies are important as they provide insights into the structure and function of the mammal and human brain. The analogy is not perfect, making human studies of AHN important in the future.

The finding that VCAE in rodents increases AHN can be questioned regarding its human analogue. First, mice have a different running pattern than humans, including short bouts of exercise followed by short breaks. Second, it is suggested that wheel running is more similar to riding a bike than running when making a human analogy (Hamilton & Rhodes, 2015). This suggestion is rather vague. What does wheel running being more similar to riding a bike mean exactly? If it is like riding a bike, then what about intensity? A bike used in low or high intensity most certainly has different effect on human beings. Third, the total distance that a rodent travels might not be comparable to humans. If the analogy of riding a bike is correct, perhaps five kilometers of bicycling is more exhausting for the human compared to five kilometers of wheel running for the rodent. Finally, the time spent exercising differs: mice run for hours each day, which humans do not. This leads to problems when making a human analogy as the majority of evidence comes from rodent studies.

The brain growing as a result of exercise is astonishing, especially contrasted to AHN decreasing as individual's age. If it was mainstream knowledge that the brain produces new brain cells and that a part of the brain becomes larger because of exercise, then motivation to exercise could be enhanced. More people motivated to exercise would lead to better health and mental health. Increased physical and mental health is an end goal in itself. Decreasing the burden on society, freeing resources to be invested in other things. Humans being healthier also affects the capacity of individuals in terms of productivity in turn generating more value in terms of money, ideas, products and services.

The aim of the thesis is also to examine how VCAE and AHN affect spatial learning and memory. The function of newly created hippocampal neurons is under debate (Gonçalves et al., 2016). Different cognitive abilities have been suggested to increase together with AHN such as contextual fear conditioning, pattern separation, and spatial learning and memory. Spatial learning and memory are the most well-established improvements from exercise in rodents (Hamilton & Rhodes, 2015).

Van Praag, Christie, et al. (1999) showed that three-month-old mice running voluntarily had an increase in spatial learning and memory compared to controls. The

mice also had an increase in AHN. It was not shown that AHN enhanced spatial learning and memory. Still, the authors of the study suggested that it was possible that the increase of AHN contributed to the increase in spatial learning and memory.

Older mice doing VCAE have been shown to have increased AHN and improved spatial learning and memory (Gibbons et al., 2014; van Praag et al., 2005). Marlatt et al. (2012) suggested that long-term VCAE led to increased BDNF, AHN and enhanced performance on the Morris water maze, indicating improved spatial learning and memory. BDNF is also upregulated in older humans who do VCAE and have an increased hippocampus (Erickson et al., 2011). VCAE is suggested to counteract the negative effects from stress on AHN, BDNF and spatial learning and memory (Yau et al., 2011). Luo et al. (2007) showed that VCAE both enhanced AHN and reversed stroke-induced spatial memory impairments.

Merritt & Rhodes (2015) examined the effects of VCAE on AHN and spatial learning and memory among five strains of mice. All five strains had an increase of AHN from running, represented as an increase in the total number of newly generated neurons in the dentate gyrus. Runners from all five strains also had enhanced spatial learning and memory compared to sedentary controls. An increase of AHN can be represented as a proportional increase compared to baseline instead of total number of newly generated neurons. This led to a different suggestion regarding the relationship between exercise-induced AHN and enhanced spatial learning and memory. Mice with the highest increase of AHN compared to baseline had the lowest enhancement on the water maze from running implying that mice with lower levels of increased AHN compared to baseline had more enhanced spatial learning and memory.

Not all studies show that VCAE both induces an increase in AHN and enhances spatial learning and memory. Rhodes et al. (2003) showed that mice bred for running had an increase of AHN until they reached a plateau. These mice did not show improvements regarding spatial learning and memory. This study was the first evidence that neurogenesis can occur without increased spatial learning and memory.

It can be concluded that VCAE increases AHN and spatial learning and memory. However, the studies imply correlation and not necessarily causation: they do not show that an increase of AHN causes increased spatial learning and memory. Clark et al. (2008) suggest that exercise-induced AHN *does* promote spatial learning and memory, implying causation. The relationship between AHN and spatial learning

and memory is still unclear. Even so, the finding that exercise promotes spatial learning and memory, with or without AHN, is very interesting.

Why does exercise increase spatial learning and memory? From an evolutionary viewpoint, it seems adaptive for humans and rodents to have an increased ability to remember where things are in the environment. It seems beneficial when exploring new territory or looking for food. It seems valid that the ability is less enhanced when rodents and humans are sedentary.

Another vital question is if AHN is linked to a healthy brain. Many things are suggested to increase AHN such as omega-3, antidepressants, curcumin and caloric restriction, learning, environmental enrichment and exercise. The broad repertoire of things that increase AHN could be speculated to mean that AHN is partly linked to brain health especially when contrasted to the things that decrease AHN such as aging, neurodegenerative disease and various substances such as alcohol, cocaine and heroin. An increase of AHN is associated with improvement in cognitive ability: contextual fear conditioning, pattern separation, spatial learning and memory as well as enhanced motor performance. In contrast, down regulation of AHN by aging, neurodegenerative disease and stress is associated with cognitive deficits. It would be interesting to explore whether AHN can be used as a measurement of brain health. If AHN could be measured non-invasively in humans, preferably at low cost, it perhaps could measure the health state of the brain. As research progresses the mapping of factors linked to AHN in humans will increase. Doing measurements of AHN could perhaps provide valuable information if an individual is at risk for neurodegenerative disease, mental health disorders or other disorders.

The field still has many questions to answer. The function of these newly generated brain cells is arguably the most important. Other areas that need to be studied further include the effect of anaerobic exercise on AHN and cognitive abilities; this have not been studied in the same degree, even though many humans are conducting anaerobic exercise. The effects of various types of exercise on AHN and cognitive functions needs to be studied further. Some types of exercise might be better in increasing AHN and cognitive function compared to others.

In the end it turns out that my teacher from Seventh Grade was wrong; the brain does generate new brain cells. Alcohol does downregulate AHN together with other things like aging and neurodegenerative disease. Now at least I know I can do

something about it; I just hope it has reached my former Seventh Grade classmates as well.

7. Conclusion

The aim of this thesis was to examine the effect of VCAE on AHN and to examine how VCAE and AHN affect spatial learning and memory, with a particular attention to rodent studies. My conclusion is that exercise has been shown to increase AHN reliably, at least in rodents. Exercise has also been shown to increase spatial learning and memory together with an increase of AHN, suggesting a correlation between exercise-induced AHN and an enhancement of exercise-induced spatial learning and memory. Research indicates that AHN is linked to both brain health and cognitive benefits. The knowledge of AHN could increase the motivation to exercise, in turn providing benefits for the individual and society at large.

References

Adams, M. M., Shi, L., Linville, M. C., Forbes, M. E., Long, A. B., Bennett, C., ... & Brunso-Bechtold, J. K. (2008). Caloric restriction and age affect synaptic proteins in hippocampal CA3 and spatial learning ability. *Experimental Neurology*, *211*(1), 141-149.

Aimone, J. B., Wiles, J., & Gage, F. H. (2006). Potential role for adult neurogenesis in the encoding of time in new memories. *Nature Neuroscience*, *9*(6), 723.

Aimone, J. B., Wiles, J., & Gage, F. H. (2009). Computational influence of adult neurogenesis on memory encoding. *Neuron*, *61*(2), 187-202.

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology*, *124*(3), 319-335.

Alvarez-Buylla, A. (1990). Mechanism of neurogenesis in adult avian brain. *Experientia*, *46*(9), 948-955.

Ang, E. T., Dawe, G. S., Wong, P. T., Moochhala, S., & Ng, Y. K. (2006). Alterations in spatial learning and memory after forced exercise. *Brain Research*, *1113*(1),186-193.

Augustinack, J. C., van der Kouwe, A. J., Salat, D. H., Benner, T., Stevens, A. A., Annese, J., ... & Corkin, S. (2014). HM's contributions to neuroscience: a review and autopsy studies. *Hippocampus*, *24*(11), 1267-1286.

Cameron, H. A., & Mckay, R. D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *Journal of Comparative Neurology*, *435*(4), 406-417.

Canales, J. J. (2016) Addiction. In Canales J. J. (Ed.), *Adult neurogenesis in the hippocampus: Health, psychopathology and brain* disease (pp. 229-248). Academic Press.

Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Reports*, *100*(2), 126.

Clark, P. J., Brzezinska, W. J., Thomas, M. W., Ryzhenko, N. A., Toshkov, S. A., & Rhodes, J. S. (2008). Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. *Neuroscience*, *155*(4), 1048-1058.

Clark, P. J., Kohman, R. A., Miller, D. S., Bhattacharya, T. K., Brzezinska, W. J., & Rhodes, J. S. (2011). Genetic influences on exercise‐induced adult hippocampal neurogenesis across 12 divergent mouse strains. *Genes, Brain and Behavior*, *10*(3), 345-353.

Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychological Science*, *14*(2), 125-130.

Conboy, L., Foley, A. G., O'Boyle, N. M., Lawlor, M., Gallagher, H. C., Murphy, K. J., & Regan, C. M. (2009). Curcumin-induced degradation of PKCδ is associated with enhanced dentate NCAM PSA expression and spatial learning in adult and aged Wistar rats. *Biochemical Pharmacology*, *77*(7), 1254-1265.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences*, *25*(6), 295-301.

Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H., & Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proceedings of the National Academy of Sciences*, *107*(5), 2367-2372.

Domínguez-Escribà, L., Hernández-Rabaza, V., Soriano-Navarro, M., Barcia, J. A., Romero, F. J., Garcí-Verdugo, J. M., & Canales, J. J. (2006) Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. *European Journal of Neuroscience*, *24*(2), 586-594.

Dong, S., Zeng, Q., Mitchell, E. S., Xiu, J., Duan, Y., Li, C., ... & Zhao, Z. (2012). Curcumin enhances neurogenesis and cognition in aged rats: *implications* for transcriptional interactions related to growth and synaptic plasticity. *PLoS One*, *7*(2), e31211.

Eisch, A. J., Barrot, M., Schad, C. A., Self, D. W., & Nestler, E. J. (2000). Opiates inhibit neurogenesis in the adult rat hippocampus. *Proceedings of the National Academy of Sciences*, *97*(13), 7579-7584.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., ... & Wojcicki, T. R. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences*, *108*(7), 3017-3022.

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), 1313.

Fabel, K., Fabel, K., Tam, B., Kaufer, D., Baiker, A., Simmons, N., ... & Palmer, T. D. (2003). VEGF is necessary for exercise‐induced adult hippocampal neurogenesis. *European Journal of Neuroscience*, *18*(10), 2803-2812.

Floresco, S. B. (2015). Spatial learning in animals. In Stolerman I.P., Price L.H. (Eds.), *Encyclopedia of Psychopharmacology* (pp. 1620-1623). Berlin: Springer.

Gamoh, S., Hashimoto, M., Hossain, S., & Masumura, S. (2001). Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. *Clinical and Experimental Pharmacology and Physiology*, *28*(4), 266-270.

Gazzaniga, M. S., Ivry, R. B., & Mangun, G. R. (2013). Cognitive Neuroscience 4th edition, New York: W. W. Norton & Company.

Gibbons, T. E., Pence, B. D., Petr, G., Ossyra, J. M., Mach, H. C., Bhattacharya, T. K., ... & Rhodes, J. S. (2014). Voluntary wheel running, but not a diet containing (−)-epigallocatechin-3-gallate and β-alanine, improves learning, memory and hippocampal neurogenesis in aged mice. *Behavioural Brain Research*, *272*, 131-140.

Gonçalves, J. T., Schafer, S. T., & Gage, F. H. (2016). Adult neurogenesis in the hippocampus: from stem cells to behavior. *Cell*, *167*(4), 897-914.

Gould, E., Beylin, A., Tanapat, P., Reeves, A., & Shors, T. J. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nature Neuroscience*, *2*(3), 260.

Gould, E., Cameron, H. A., Daniels, D. C., Woolley, C. S., & McEwen, B. S. (1992). Adrenal hormones suppress cell division in the adult rat dentate gyrus. *Journal of Neuroscience*, *12*(9), 3642-3650.

Gould, E., Tanapat, P., McEwen, B. S., Flügge, G., & Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proceedings of the National Academy of Sciences*, *95*(6), 3168-3171.

Hamilton, G. F., & Rhodes, J. S. (2015). Animal models of exercise-brain interactions. In McMorris, T. (Ed.), *Exercise-cognition interaction: Neuroscience perspectives* (pp. 43-63). Academic Press.

Hartfuss, E., Galli, R., Heins, N., & Götz, M. (2001). Characterization of CNS precursor subtypes and radial glia. *Developmental Biology*, *229*(1), 15-30.

He, C., Qu, X., Cui, L., Wang, J., & Kang, J. X. (2009). Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. *Proceedings of the National Academy of Sciences*, *106*(27), 11370-11375.

Herrera, D. G., Yagüe, A. G., Johnsen-Soriano, S., Bosch-Morell, F., Collado-Morente, L., Muriach, M., ... & García-Verdugo, J. M. (2003). Selective impairment of hippocampal neurogenesis by chronic alcoholism: protective effects of an antioxidant. *Proceedings of the National Academy of Sciences*, *100*(13), 7919-7924.

Hillman, C. H., Erickson, K. I., & Kramer, A. F. (2008). Be smart, exercise your heart: exercise effects on brain and cognition. *Nature Reviews Neuroscience*, *9*(1), 58.

Höglinger, G. U., Rizk, P., Muriel, M. P., Duyckaerts, C., Oertel, W. H., Caille, I., & Hirsch, E. C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nature Neuroscience*, *7*(7), 726.

Innis, S. M. (2007). Dietary (n-3) fatty acids and brain development. *The Journal of Nutrition*, *137*(4), 855-859.

Jin, X. (2016). The role of neurogenesis during development and in the adult brain. *European Journal of Neuroscience*, *44*(6), 2291-2299.

Kawakita, E., Hashimoto, M., & Shido, O. (2006). Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience*, *139*(3), 991-997.

Keilhoff, G., Bernstein, H. G., Becker, A., Grecksch, G., & Wolf, G. (2004). Increased neurogenesis in a rat ketamine model of schizophrenia. *Biological Psychiatry*, *56*(5), 317-322.

Kempermann, G., & Gage, F. H. (2002). Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. *European Journal of Neuroscience*, *16*(1), 129-136.

Kim, B. K., Shin, M. S., Kim, C. J., Baek, S. B., Ko, Y. C., & Kim, Y. P. (2014). Treadmill exercise improves short-term mem- ory by enhancing neurogenesis in amyloid beta-induced Alzheimer disease rats. *Journal of Exercise Rehabilitation*, *10*, 2–8.

Kobilo, T., Liu, Q. R., Gandhi, K., Mughal, M., Shaham, Y., & van Praag, H. (2011). Running is the neurogenic and neurotrophic stimulus in environmental enrichment. *Learning & Memory*, *18*(9), 605-609.

Kolb, B., & Gibb, R. (2014). Searching for the principles of brain plasticity and behavior. *Cortex*, *58*, 251-260.

Kolb, B., & Whishaw, I. Q. (1998). Brain plasticity and behavior. *Annual Review of Psychology*, *49*(1), 43-64.

Kornack, D. R., & Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proceedings of the National Academy of Sciences*, *96*(10), 5768-5773.

Kronenberg, G., Bick-Sander, A., Bunk, E., Wolf, C., Ehninger, D., & Kempermann, G. (2006). Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. *Neurobiology of Aging*, *27*(10), 1505-1513.

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *Journal of Neuroscience*, *16*(6), 2027-2033.

Lee, J., Duan, W., Long, J. M., Ingram, D. K., & Mattson, M. P. (2000). Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. *Journal of Molecular Neuroscience*, *15*(2), 99-108.

Lee, M. C., Inoue, K., Okamoto, M., Liu, Y. F., Matsui, T., Yook, J. S., & Soya, H. (2013). Voluntary resistance running induces increased hippocampal neurogenesis in rats comparable to load-free running. *Neuroscience Letters*, *537*, 6-10.

Lee, J., Seroogy, K. B., & Mattson, M. P. (2002). Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *Journal of Neurochemistry*, *80*(3), 539-547.

Li, H., Liang, A., Guan, F., Fan, R., Chi, L., & Yang, B. (2013). Regular treadmill running improves spatial learning and memory performance in young mice through increased hippocampal neurogenesis and decreased stress. *Brain Research*, *1531*, 1–8.

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., & Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature*, *484*(7394), 381.

Lledo, P. M., Alonso, M., & Grubb, M. S. (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nature Reviews Neuroscience*, *7*(3), 179.

Lois, C., & Kelsch, W. (2014). Adult neurogenesis and its promise as a hope for brain repair. *Frontiers in Neuroscience*, *8*, 165.

Lucassen, P. J., Meerlo, P., Naylor, A. S., Van Dam, A. M., Dayer, A. G., Fuchs, E., ... & Czeh, B. (2010). Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. *European Neuropsychopharmacology*, *20*(1), 1-17.

Luo, C. X., Jiang, J., Zhou, Q. G., Zhu, X. J., Wang, W., Zhang, Z. J., ... & Zhu, D. Y. (2007). Voluntary exercise‐induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. *Journal of Neuroscience Research*, *85*(8), 1637-1646.

Malberg, J. E., Eisch, A. J., Nestler, E. J., & Duman, R. S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *Journal of Neuroscience*, *20*(24), 9104-9110.

Man Lau, B. W., Yau, S. Y., Po, K. T., & So, K. F. (2016) Neurological Disorders. In Canales J.J. (Ed.), *Adult neurogenesis in the hippocampus: Health, psychopathology and brain disease* (pp. 229-248). Academic Press.

Marlatt, M. W., Potter, M. C., Lucassen, P. J., & van Praag, H. (2012). Running throughout middle‐age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. *Developmental Neurobiology*, *72*(6), 943-952.

Merritt, J. R., & Rhodes, J. S. (2015). Mouse genetic differences in voluntary wheel running, adult hippocampal neurogenesis and learning on the multi-strain-adapted plus water maze. *Behavioural Brain Research*, *280*, 62-71.

Molina-Navarro, M. M., & García-Verdugo, J. M. (2016). Neurobiology. In *Adult Neurogenesis in the Hippocampus: Health, psychopathology and brain disease* (pp. 1-17).

Murphy, T., & Thuret, S. (2016). Dietary and Nutritional Regulation. In *Adult Neurogenesis in the Hippocampus: Health, psychopathology and brain disease* (pp. 109-143).

Mustroph, M. L., Chen, S., Desai, S. C., Cay, E. B., DeYoung, E. K., & Rhodes, J. S. (2012). Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. *Neuroscience*, *219*, 62-71.

Nieto-Estévez, V., Defterali, Ç., & Vicario-Abejón, C. (2016). IGF-I: a key growth factor that regulates neurogenesis and synaptogenesis from embryonic to adult stages of the brain. *Frontiers in Neuroscience*, *10*, 52.

Nokia, M. S., Lensu, S., Ahtiainen, J. P., Johansson, P. P., Koch, L. G., Britton, S. L., & Kainulainen, H. (2016). Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained. *The Journal of Physiology*, *594*(7), 1855-1873.

Noonan, M. A., Choi, K. H., Self, D. W., & Eisch, A. J. (2008). Withdrawal from cocaine self-administration normalizes deficits in proliferation and enhances maturity of adult-generated hippocampal neurons. *Journal of Neuroscience*, *28*(10), 2516-2526.

O'reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade‐off. *Hippocampus*, *4*(6), 661-682.

Pereira, A. C., Huddleston, D. E., Brickman, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., ... & Small, S. A. (2007). An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proceedings of the National Academy of Sciences*, *104*(13), 5638-5643.

Rebar, A. L., Stanton, R., Geard, D., Short, C., Duncan, M. J., & Vandelanotte, C. (2015). A meta-meta-analysis of the effect of physical activity on depression and anxiety in non-clinical adult populations. *Health Psychology Review*, *9*(3), 366-378.

Rethorst, C. D., Wipfli, B. M., & Landers, D. M. (2009). The antidepressive effects of exercise. *Sports Medicine*, *39*(6), 491-511.

Rhodes, J. S., van Praag, H., Jeffrey, S., Girard, I., Mitchell, G. S., Garland, T., & Gage, F. H. (2003). Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behavioral Neuroscience*, *117*(5), 1006.

Sahay, A., Scobie, K. N., Hill, A. S., O'carroll, C. M., Kheirbek, M. A., Burghardt, N. S., ... & Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature*, *472*(7344), 466.

Seo, D. Y., Lee, S. R., Kim, N., Ko, K. S., Rhee, B. D., & Han, J. (2014). Humanized animal exercise model for clinical implication. *Pflügers Archiv-European Journal of Physiology*, *466*(9), 1673-1687.

Smith, P. J., Blumenthal, J. A., Hoffman, B. M., Cooper, H., Strauman, T. A., Welsh-Bohmer, K., ... & Sherwood, A. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosomatic Medicine*, *72*(3), 239.

Simpson, J. M., Gil-Mohapel, J., Pouladi, M. A., Ghilan, M., Xie, Y., Hayden, M. R., & Christie, B. R. (2011). Altered adult hippocampal neurogenesis in the YAC128 transgenic mouse model of Huntington disease. *Neurobiology of Disease*, *41*(2), 249-260.

Sinclair, D. A. (2005). Toward a unified theory of caloric restriction and longevity regulation. *Mechanisms of Ageing and Development*, *126*(9), 987-1002.

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., ... & Possnert, G. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell*, *153*(6), 1219-1227.

Stubbs, B., Vancampfort, D., Rosenbaum, S., Firth, J., Cosco, T., Veronese, N., ... & Schuch, F. B. (2017). An examination of the anxiolytic effects of exercise for people with anxiety and stress-related disorders: a meta-analysis. *Psychiatry Research*, *249*, 102-108.

Taliaz, D., Stall, N., Dar, D. E., & Zangen, A. (2010). Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Molecular Psychiatry*, *15*(1), 8

Trejo, J. L., 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. *Journal of Neuroscience*, *21*(5), 1628-1634.

Van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences*, *96*(23), 13427-13431.

Van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature neuroscience*, *2*(3), 266.

Van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *Journal of Neuroscience*, *25*(38), 8680-8685.

Verret, L., Jankowsky, J. L., Xu, G. M., Borchelt, D. R., & Rampon, C. (2007). Alzheimer's-type amyloidosis in transgenic mice impairs survival of newborn neurons derived from adult hippocampal neurogenesis. *Journal of Neuroscience*, *27*(25), 6771-6780.

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), 848.

Voss, M. W., Vivar, C., Kramer, A. F., & van Praag, H. (2013). Bridging animal and human models of exercise-induced brain plasticity. *Trends in Cognitive Sciences*, *17*(10), 525-544.

Wojtowicz, J. M., Askew, M. L., & Winocur, G. (2008). The effects of running and of inhibiting adult neurogenesis on learning and memory in rats. *European Journal of Neuroscience*, *27*(6), 1494-1502.

Yau, S. Y., Lau, B. W. M., Tong, J. B., Wong, R., Ching, Y. P., Qiu, G., ... & So, K. F. (2011). Hippocampal neurogenesis and dendritic plasticity support running-improved spatial learning and depression-like behaviour in stressed rats. *PLoS One*, *6*(9), e24263.