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Effects of D-Galactose Treatment and Moderate Exercise on Spatial Memory in Rats

Amanda R. Austin

Western Michigan University, araustin084@gmail.com

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EFFECTS OF D-GALACTOSE TREATMENT AND MODERATE EXERCISE ON SPATIAL MEMORY IN RATS

by

Amanda R. Austin

A Dissertation
Submitted to the Faculty of The Graduate College in partial fulfillment of the requirements for the Degree of Doctor of Philosophy Department of Psychology Advisor: Lisa Baker, Ph.D.

Western Michigan University
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EFFECTS OF D-GALACTOSE TREATMENT AND MODERATE EXERCISE ON SPATIAL MEMORY IN RATS

Amanda R. Austin, Ph.D.
Western Michigan University, 2012

Cognitive decline is a process frequently associated with aging. Physical exercise appears to counteract cognitive decline, specifically spatial abilities, and decreases the effects of reactive oxygen species (ROS) associated with aging. In addition, brain derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF) are well recognized as chemical mediators of the neurophysiological benefits of exercise.

In order to study the impact of exercise on spatial memory and neurotrophic factors, this study utilized an animal model of accelerated aging involving chronic d-galactose administration. Specifically, previous research indicates daily injections of d-galactose for 6-10 weeks may increase ROS in mice and produce significant deficits in learning and memory. The current study investigated d-galactose's ability to simulate aging in rats. This study also further investigated the interaction between exercise, spatial abilities, and levels of BDNF and GDNF protein content within the hippocampus and pre-frontal cortex.

Forty-eight male Sprague-Dawley rats were randomly assigned to receive once daily injections of 100 mg/kg d-galactose (n=24) or saline (n=24) for a period of eight weeks. Half the animals in each group were also subjected to a moderate forced exercise regimen utilizing running wheels during the same eight week period (13 m/min, 30 min,
3 days per week), while the remaining rats were exposed to the apparatus for the same amount of time, but with the wheels turned off. Immediately following completion of the eight week injection and exercise regimen, rats underwent a 19 day radial arm maze (RAM) procedure. This procedure consisted of two trials per day with a one hour inter-trial interval. Subsequently, rats were euthanized, brains were removed, and prefrontal cortex and hippocampal tissues were dissected and processed for analysis of BDNF and GDNF protein content using the ELISA assay. The results indicate spatial navigation performance was not significantly altered by d-galactose or by exercise. Furthermore, BDNF and GDNF protein content in the hippocampus or pre-frontal cortex did not differ among treatment groups. Several possible reasons for negative findings are addressed.
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Amanda R. Austin
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INTRODUCTION

The aging process is frequently associated with several changes, some inevitable, that span from benign nuisances (e.g. increased fatigue) to detrimental, life-altering events (e.g. diseases that negate independent living). Cognitive decline is a process frequently associated with aging (Hansalik, Skalicky, & Viidik, 2006; Deslandes et al., 2009; O’Callaghan, Griffin, & Kelly, 2009; Witte, Fobker, Gellner, Knecht, & Floel, 2009). The prevalence of neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease, increase with age (Deslandes et al., 2009). Research indicates that approximately 80% of adults 65 years or older have at least one chronic or neurodegenerative disease. In addition, 50% of the same population has two or more chronic or neurodegenerative diseases (Omodei & Fontana, 2011). Neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease, are frequently associated with cognitive decline. One of the various symptoms experienced by patients with Alzheimer’s disease is trouble understanding visual images and spatial relationships (Alzheimer’s Association, 2012). In addition, late stages of Parkinson’s disease are associated with cognitive deficits, such as decreased attention, memory, and visuo-spatial functioning (Emre et al., 2007).

Spatial memory decline is not just a symptom of neurodegenerative diseases. Declines in spatial memory are also associated with the normal aging process (Deslandes et al., 2009; O’Callaghan et al., 2009; Stranahan, Zhou, Martin, & Maudsley, 2009; Witte et al., 2009). It is well documented that the hippocampus is important for spatial memory, and memory in general (Griffin et al., 2009; O’Callaghan et al., 2009; Stranahan et al., 2009; Yuede et al., 2009). To attenuate the effects of age, as well as neurodegenerative
diseases, research has concentrated on addressing hypotheses regarding why cognitive decline occurs with aging, as well as ways to ameliorate or lessen the effects of old age on memory.

**Cellular Mechanisms of Aging**

One of the first proposed theories of aging is the oxidative stress theory, originally known as the free radical theory. This theory was first proposed in the 1950s by Denham Harman (Perez et al., 2009). This theory correlates reactive oxygen species (ROS) with the process of aging, suggesting that oxidative stress increases cellular damage. Specifically, it is indicated that ROS affect macromolecules, such as lipids and proteins, and DNA.

The mitochondria theory of aging is a recent theory that extends the core concepts of the oxidative stress theory (Jacobs, 2003). This theory is based upon a cycle in which somatic mutation of mitochondrial DNA (mtDNA) engenders respiratory chain dysfunction, which enhances the production of DNA-damaging oxygen radicals. Similar to the oxidative stress theory, the resulting accumulation of mtDNA mutations leads to tissue dysfunction and degeneration of tissues, and possibly neurodegenerative diseases.

It has also been indicated that the life span of an organism is determined by the level of oxidative damage, thus a large increase in oxidative damage will shorten the organisms’ lifespan (Lee, Duan, Long, Ingram, & Mattson, 2001). The decrease in lifespan is associated with a gradual decline in cellular, and eventually, tissue function (Guarente, 2008). In a study conducted by Sohal, Ku, Agarwal, Forster, & Lal (1994), the authors sought to test the relevance and validity of the oxidative stress hypothesis of aging on lifespan. Fifty-nine male C57BL/6NNia mice aged 9, 16, or 23 months were
used. The authors assessed protein carbonyl content, mitochondrial oxygen consumption, and levels of ROS, superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$), within the kidney, heart, and brain. The results indicated that protein carbonyl content, a common marker for protein oxidation, mitochondrial oxygen consumption, and levels of ROS significantly increased in all organs with age. The authors also reported an increase in mortality rate with age. Overall, both theories of aging hypothesize that ROS contribute to the process of aging and are correlated with neurodegenerative disease (Lee, Lin, Boelsterli, & Chung, 2009).

**D-galactose: A Model of Aging**

Assessing the effects of aging on an organism poses certain challenges. One of these challenges is the increase in frequency of animal deaths with increasing age. In a study that specifically assessed lifespan of male Sprague Dawley rats ($N = 747$ rats) (Jones & Kimeldorf, 1963), it was reported that lifespan ranged from 20 – 26 months. The authors also reported a 50% survival rate at 23 months, and the highest percentage of deaths occurring between 18 – 29 months. Due to the high mortality rate, low survival rate, and the minimum number of rats needed to run statistics, assessing the effects of old age is difficult unless one has a large number of rats at the beginning of a study.

Several solutions have been attempted to address this challenge. One solution is the creation and implementation of mutant animal strains. For example, several mutant rodent models for Alzheimer’s disease have been developed, such as 3xTgAd mice, a triple mutant mouse model that expresses amyloid precursor protein, presenilin-1, and tau mutations (Halogapppa et al., 2007). Mutant models that specifically alter learning and memory were also created. Specifically, the P8 mouse model is a mutant mouse strain
that exhibits impaired learning and memory function early in life. This strain also has a relatively short lifespan in comparison to other mouse strains (Komatsu et al., 2008). The first disadvantage to mutant animal strains is possible shortening of lifespan. For example, the P8 mouse model provides impaired learning and memory early in life, but the shortened lifespan limits investigations of the effects of old age, or extreme old age, upon learning and memory function. A second disadvantage is the high cost per animal. Typically, a mutant animal strain will cost more per animal than a standard lab strain. This increase in price is normally due to the genetic manipulations performed to gain the desired genetic traits.

An alternative more cost effective approach involves the implementation of chemical substances that accelerate aging. D-galactose is a chemical substance used in the past several decades that presumably accelerates aging processes. Moreover, it has been indicated in specifically affecting spatial memory (Kim et al., 2004; Wei, Li, Song, Ai, Chu, & Li, 2005; Zhang, Li, Cui, & Zuo, 2005). Researchers in China serendipitously stumbled upon this substance’s ability as a model of aging while testing sub-acute toxicity of several carbohydrates. The researchers reported that d-galactose induced neurological impairments in rodents, shortened animal lifespan (Song, Bao, Li, & Li, 1999), and caused neurodegeneration (Cui et al., 2004). Further research indicates that changes induced by d-galactose resemble changes observed in the normal aging process. These changes include decreased neuromuscular activity, increased production of free radicals, deceased antioxidant enzyme activity, and diminished immune responses (Song et al., 1999).
Since the initial discovery, researchers have used d-galactose as a model of aging and conducted multiple studies to determine how d-galactose ages animals. To begin, d-galactose is a reducing sugar that reacts with free amines of amino acids in proteins to form advanced glycation end-products (AGEs) through nonenzymatic glycation (Parameshwaran, Irwin, Stellou, & Pinkert, 2010). Nonenzymatic glycation is believed to contribute to the process of aging through the slow modification of structural, functional, and biochemical parameters of proteins and DNA, including mtDNA. The modifications are mediated by the formation of AGEs (Sensi, Pricci, Andreani, & Mario, 1991).

A second way in which nonenzymatic glycation contributes to aging is through the association of AGEs and the direct production of ROS. At normal levels, d-galactose may not have an adverse effect on the body, and is usually converted into glucose; however, at high levels, it can be oxidized into $\text{H}_2\text{O}_2$, which through further reaction with lipids and proteins can produce aldehydes (Lei, Hua, Xiao, Ding, Hang, & Hu, 2008). The accumulation of $\text{H}_2\text{O}_2$ and aldehydes may initiate mitochondrial dysfunction leading to intracellular damage (Parameshwaran et al., 2010). Song et al. (1999) conducted a study to investigate the link between d-galactose and AGEs. Five-month-old female C57BL/6J mice were subcutaneously injected with 50 mg/kg d-galactose for 8 weeks. The authors indicated that young mice treated with d-galactose had an increased level of AGEs when compared to young control mice. In addition, the authors reported that young mice treated with d-galactose had decreased spontaneous motor activity, increased memory errors in passive avoidance, decreased lymphocyte proliferation and IL-2 production, and decrease in superoxide dismutase activity when compared to controls (1999).
Overall, d-galactose is closely related to the process of aging through nonenzymatic glycation and the production of AGEs. The effects of glycation and AGEs are similar to those accounted for by oxidative stress and mitochondrial damage. Glycation can alter the structure and function of DNA and mtDNA (Kong, Wang, Wang, Hu, & Liu, 2006; Long et al., 2007; Sensi et al., 1991), thus having similar outcomes to that predicted by the mitochondrial theory of aging. AGEs can produce ROS which causes tissue dysfunction, indicating similar outcomes to that predicted by the oxidative stress theory of aging.

**D-Galactose’s Effects on Cellular Processes and Learning and Memory**

In the past decade, research on d-galactose has focused on its mechanism of action within the brain and its effects on learning and memory. As discussed above, oxidative stress induced by d-galactose is thought to cause several detrimental effects, such as cognitive decline. Further support for this hypothesis is research on oxidative stress biomarkers within the brain.

Common *in vivo* oxidative stress biomarkers include malondialdehyde (MDA), total antioxidative capabilities (T-AOC), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px) (Zhang, Li, Cui, Zuo, 2005). MDA is a measurement of the amount of ROS within the organism, while T-AOC, T-SOD, and GSH-Px measure the level of enzymes that inactivate free radicals. Measuring biomarkers is one way to determine d-galactose’s mechanism of action. Cui et al. (2006) conducted a study in which they investigated the effects of chronic d-galactose exposure on oxidative damage in mice. Adult male C57BL/6 mice were administered subcutaneous injections of 100 mg/kg d-galactose once daily for seven weeks. After the completion of the seventh week,
blood was collected and used to analyze MDA, T-AOC, T-SOD, and GSH-Px levels within serum. The authors reported an increase in MDA combined with a decrease in the activities of T-AOC, T-SOD, and GSH-Px in d-galactose treated mice when compared to controls. These results indicate that d-galactose increased oxidative stress by inhibiting the function of enzymes which impede and break down of ROS within mice.

In respect to learning and memory, evidence suggests that chronic injection of d-galactose for 6 – 10 weeks causes dysfunction of astrocytes, neurodegeneration, impairment of neurogenesis, and decline in spatial memory (Cui et al., 2006). Research with astrocytes is one of several ways to study neurodegeneration, specifically the neurodegeneration associated with Alzheimer’s disease. Astrocytes are the most abundant glial cell type within the brain, and they have an important role in regulation of synaptic network formation and neural electrical activity, as well as provide neuronal protection (Lei, Hua, Xiao, Ding, Hang, & Hu, 2008). In a high ROS environment, such as an organism chronically injected with d-galactose, astrocytes undergo subcellular changes. These changes include hypertrophy where swollen processes eventually engulf proximal synaptic structures, thus compromising the surrounding tissue (2008). A second way to investigate neurodegeneration is to quantify apoptotic cells. In a study conducted by Cui et al. (2006) male C57BL/6 mice were administered subcutaneous injection of 100 mg/kg d-galactose once daily for seven weeks. After, tissue was excised and processed using hematoxylin and eosin (H&E) staining and a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The authors reported that four sections of the hippocampal formation (CA1, CA2, CA3, and dentate gyrus) all had increases in
pyknotic nuclei, nuclei of cells undergoing apoptosis, and TUNEL positive cells, or cells with DNA degradation.

The hippocampus is the primary area in the adult brain where neurogenesis occurs (Kim et al., 2004; Zhang, Li, Cui, & Zuo, 2005). Activities involving new learning and exercise can increase neurogenesis within an organism, thus increasing learning and memory capabilities, including spatial memory. This process can either build upon the neurons that already exist or they may replace any that were destroyed due to environmental factors. Neurogenesis is a process that declines with age and can also be accelerated by oxidative stress. In a study conducted by Zhang et al. (2005), male C57BL/6 mice were administered subcutaneous injection of 100 mg/kg d-galactose once daily for seven weeks. The results indicated a decrease in cell proliferation and long-term survival of newly born cells within the dentate gyrus.

Another study investigated the effects of similar doses of d-galactose on spatial memory in C57 mice. Specifically, Wei, Li, Song, Ai, Chu, & Li (2005) investigated the effects of d-galactose on learning in a Morris Water Maze (MWM). Young female C57 mice were administered subcutaneous injections of 50, 100, or 200 mg/kg d-galactose once daily for eight weeks. The MWM procedure consisted of four day learning and memory training followed by a probe trial on day five. Each training day consisted of a morning block and afternoon block, with each block consisting of two 60 s trials. The results indicated increased escape latencies in the 100 and 200 mg/kg group, but not the 50 mg/kg group, when compared to controls. In addition, mice treated with 100 or 200 mg/kg swam significantly less time and distance in the target quadrant during the probe trial. These results indicate a deficit in spatial memory. These two studies together
indicate the debilitating effects of d-galactose upon spatial memory, as well as the attenuation of neurogenesis in the dentate gyrus of the hippocampus, an area that normally contributes to spatial memory capabilities.

In summary, d-galactose is a substance previously shown to induce cellular changes within an organism that resemble natural aging (Cui et al., 2006; Chen, Zhong, Peng, Sun, & Kong, 2010). The deleterious effects of d-galactose have been attributed to an increase in oxidative stress, as well as the inhibition of systems responsible for decreasing ROS within the body (Zhang, Li, Cui, & Zuo, 2005; Cui et al., 2006; Hua, Lei, Zhang, Ding, Han, Hu, Xiao, 2007; Lei et al., 2008). Furthermore, the systems typically affected include brain regions that contribute directly or indirectly to the processes of learning and memory (Lei et al., 2008). To date, the published research on d-galactose treatment as a model of accelerated aging has utilized mice. Presumably d-galactose will have similar effects in other rodents, although this has not yet been determined.

**Forced Exercise**

It is well established that exercise is beneficial to humans, having influences in all organs and systems of the body. Though the effects of physical exercise on health are thoroughly researched, its effects on all areas of the body have not been extensively studied. In the past several decades, research has focused on the central nervous system, particularly areas directly involved in learning and memory (Braszko, Kaminski, Hryszko, Jedynak, Brzosko, 2001). Current literature indicates that physical exercise appears to have an impact on the effects of aging and age-related diseases, whether
chronic or neurodegenerative (Alaei, Moloudi, Sarkaki, Azizi-Malekagadi, & Hanninen, 2007).

Among the most important impacts of exercise is its effect on oxidative stress. Two studies best summarize these effects, with the first being conducted by Navarro, Gomez, Lopez-Cepero, & Boveris (2003). In this study male and female mice from the CD-1 strain were moderately exercised. At 28 weeks of age, the mice were subjected to treadmill training at 10, 15, and 20 cm/s for 5 min each day, seven days a week, for 50 weeks total. The authors analyzed tissue from the brain, heart, liver, and kidney at 52 and 78 weeks of age. The results indicate that levels of oxidative stress, levels of MDA specifically, in non-exercise rats were increased by 20 - 39% at week 52, and by 45 - 55% at week 78. In addition, protein carbonyls increased by 26 – 43% at week 52, and by 50 – 63% at week 78. Moderate exercise prevented the increase of MDA and protein carbonyls at week 52; however, the effect was lost at 78 weeks of age. The authors also reported the effects of moderate exercise on levels of antioxidant enzymes, specifically Mn-SOD, Cu,Zn-SOD, and catalase. It was reported that non-exercise mice had a decrease of 14 – 18% at week 52, and 31 – 38% decrease at week 78. Moderate exercise prevented the decrease in activity at week 52 by 67 – 98%, but the effects were reduced to 7 – 9% at week 78.

In the second study (Ogonovszky et al., 2005), twenty-eight Wistar rats were exposed to either moderate training, strenuous training, or over training. Moderate training consisted of 1 h of swimming per day, five days a week, for eight weeks total. Strenuous training consisted of a swimming duration that increased by 30 min each week until it was 4.5 h in duration. The swimming regimen also lasted for eight weeks total.
Over-training consisted of 1 h of swimming per day, five days a week, for six weeks, with an abrupt increase of duration to 4.5 h for the remaining two weeks. The authors reported that over-training significantly increased proteasome activity, an enzyme that is primarily responsible for repair of oxidatively modified proteins, within the brain. This effect did not occur in control, moderately trained, or strenuously trained rats.

Another effect of exercise is to decrease neurodegeneration (Hoveida, Alaei, Oryn, Parivar, & Reisi, 2011) and apoptosis (Uysal, et al., 2005; Chae & Kim, 2009). In a study conducted by Hoveida et al. (2011), a rodent model of Alzheimer’s disease was used, utilizing lesions to the nucleus basalis magnocellularis. Prior to receiving lesions, rats were trained to locate a submerged platform in a MWM. The spatial task consisted of two phases, acquisition phase and a probe trial. During the acquisition phase, male Wistar rats learned to locate the submerged platform. Each rat was subjected to four 60 s trials per day for four consecutive days. After completing the acquisition phase, rats were anesthetized for lesion or sham surgery. After surgery, rats were subjected to forced treadmill exercise at 17 m/min, 60 min daily, for 60 days total. To determine the functional effects of neurodegeneration, the authors assessed speed and distance swam during the acquisition phase, as well as retention of platform location during the two probe trails, one 30 days after surgery and the other 60 days after surgery. The authors reported no statistical difference between sham and lesions group for escape latency and distance traveled during the acquisition phase. However, during the probe trial 60 days after surgery, the lesion-exercise group performed significantly better than the lesion-rest group. This indicates that exercise attenuated any further damage due to lesions, whereas the rest group continued to accumulate damage which affected spatial performance.
Chae & Kim (2009) investigated the effects of moderate-intensity treadmill exercise on apoptosis in the hippocampus of male Sprague-Dawley rats. Rats were subjected to exercise five days a week for eight weeks. During the first five weeks of the exercise regimen, speed and duration were gradually increased from 10 – 12 m/min for 10 min to 18 – 20 m/min for 50 min. Subsequently, tissue was extracted and processed. The authors reported a high level of TUNEL positive cells in the non-exercise group, and a significant decrease in the exercise group.

Furthermore, exercise has been indicated as a neuroprotective agent that enhances neurogenesis (Kim et al., 2004; Radak et al., 2011) in the hippocampus. In a study conducted by Kim et al. (2004), young (8-week-old) and adult (62-week-old) male Sprague-Dawley rats were subjected to forced treadmill exercise for 30 min once a day for five consecutive days. The 30 min consisted of 3 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the last 20 min. In control rats, the 4-week-old control rats had the most active cell proliferation within the dentate gyrus. This effect was not observed in the 8-week-old or 62-week-old control groups. However, in the exercise group, cell proliferation occurred in all age groups, with the most prevalent increase occurring in the 8-week-old group.

**Effects of Forced Exercise on Learning and Memory**

Regardless of the cellular mechanisms of action (reduced ROS or neurodegeneration, or enhanced neurogenesis), forced exercise has been indicated in counteracting cognitive decline, specifically spatial abilities (Fordyce & Wehner, 1993; Braszko, Kaminski, Hryszko, Jedynak, & Brzosko, 2001; Albeck, Sano, Prewitt, & Dalton, 2006; Alaei et al., 2007; O’Callaghan, Ohle, & Kelly, 2007; Alaei, Moloudi, &
Sarkaki, 2008; Chae & Kim, 2009; Deslandes et al., 2009; Griffen, Bechara, Birch & Kelly, 2009; O'Callaghan et al., 2009; Stranahan et al., 2009). Reconciling the results of previous studies on the neurobehavioral benefits of forced exercise is somewhat complicated by the fact that the intensity of exercise varies considerably among these studies. In the literature, exercise is operationally defined as mild, moderate, and vigorous. In addition, the behavioral assay used to assess spatial memory performance varies among studies. The most widely used spatial navigation task is the Morris Water Maze (MWM). Other spatial memory tasks include object recognition/substitution, and the radial arm maze (RAM). The results of three representative studies are summarized below to illustrate examples of research on the performance improving effects of mild, moderate, and strenuous exercise.

To begin, mild intensity forced exercise is analogous to walking. In a study by Albeck et al. (2006) 23-month-old male Brown Norway/Fisher 322 were subjected to mild forced treadmill exercise. Rats were exercised at a speed of 8 m/min, 15 min each day, five days a week, for seven weeks total. Immediately after completing the exercise regimen, rats began training in the MWM. Each rat completed four 60 s trials per day with an inter-trial interval (ITI) of 15 min for seven consecutive days. During these seven days, the platform was submerged. On the eighth day, the authors tested the animals with a visible platform. This was to ensure that the rats did not have any sensory or motor deficits. Rats completed four trials, and for each trial the platform was moved to a new location within the maze. Results indicated that mild forced exercise significantly shortened escape latency during the hidden platform trials when compared to the sedentary group. In addition, exercised rats had significantly shorter path lengths. During
the visible platform trials, there was no significant difference between exercised and sedentary rats. These results indicate that the difference in spatial memory was not due to effects of age, sensory or motor issues, but were attributed to the exercise regimen. Overall, mild exercise does appear to have an impact upon spatial memory. However, few studies have investigated the effects of mild exercise. The majority of forced exercise studies have investigated moderate or vigorous exercise regimens.

Moderate exercise is akin to jogging. Studies of exercise intensity have varied intensity between 10 m/min to 19 m/min. Griffin et al. (2009) subjected male Wistar rats to treadmill exercise for seven consecutive days, 1 h per day, fluctuating between 10 – 15 m/min. Once the regimen was completed, rats were subjected to either object displacement or object substitution learning. For the learning task, the apparatus consisted of a black circular open field. Rats were first habituated to the apparatus for two days. Subsequently, objects were placed in the apparatus and the rats were allowed to freely explore. The authors measured active exploration, where rats were at least touching the object with their nose. The dependent measure used for training was time spent exploring each object, and the dependent measure for testing was either time spent exploring a moved or novel object. All measures were expressed as a percentage of total exploration time in seconds. Object displacement was used to assess spatial recognition memory. During the training phase, three objects were positioned in the open field and a spatial cue was fixed to the wall. Rats in this group were either allowed to explore objects for three 5 min trials with an ITI of 5 min, or a single 5 min trial. The testing phase consisted of one object being moved from its original position. Object substitution was used to assess non-spatial recognition memory. During training, three objects were
positioned in the open field. Each rat was allowed to explore the objects for three 5 min trials. In addition, some rats received overtraining. Rats in overtraining received nine 5 min trails. During the testing phase, one object was replaced with a novel object. Rats received a single 5 min trial. Results indicate that one week of forced exercise failed to enhance exploration of a displaced object when several training trials were received; however, during the training where rats were exposed once to the objects, exercised rats demonstrated significantly greater exploration of the displaced object when compared to sedentary rats. In the object substitution task, exercised rats displayed significantly greater exploration of the novel object when compared to sedentary rats.

Alaei et al. (2007) also investigated the effects of moderate exercise. The exercise regimen consisted of 1 h at 17 m/min for 38 days, with the last eight days including MWM training. Rats were trained for 8 consecutive days, with four trials each day, and each trial separated by a 5 min ITI. The authors reported an effect of exercise on spatial memory. Specifically, exercise rats had shorter escape latencies and shorter swim paths in the MWM when compared to controls.

Vigorous exercise is similar to running. In the animal literature, vigorous exercise is defined as any forced exercise regimen greater than 19 m/min. Ang et al. (2006) investigated the effects of learning and memory in male Wistar rats after 12 weeks of forced treadmill running. During the first week, speed was increased from 5 to 30 m/min, and duration was increased from 10 to 50 min over five days. For the remaining 11 weeks, speed and duration was maintained at 30 m/min for 50 min. After completing the regimen, rats were exposed to the MWM. During training, each rat received four 60 s trials per day for two days. For the first test, each rat received three blocks of four trials
separated by a 30 min ITI. The second test was a probe trial, and was conducted 30 min after the last trial of the first test. During the probe trial, each rat received one 60 s trial. The authors reported a significant decrease in escape latency and distance swam in the exercise group. This result is even more significant since rats in the exercise group had significantly slower swim speeds than non-exercised rats. Lastly, exercised rats spent a higher percentage of time in the correct quadrant than non-exercised rats.

Although all three exercise intensities (mild, moderate, and vigorous) have a positive effect on the brain and spatial memory, little is known about the minimum duration, or minimum intensity level, needed to achieve these benefits. Currently, health organizations state that adults (age 18 – 64) and elderly (age 65+) should engage in 150 min of moderate-intensity aerobic activity and muscle strengthening for two or more days per week. For greater health benefits, individuals should receive 300 min of moderate-intensity aerobic activity and two or more days of muscle strengthening per week (Center for Disease Control and Prevention, 2011; Mayo Clinic, 2012; US Department of Health and Human Services, 2012; World Health Organization, 2012). Aerobic activity of this level is indicated to decrease excess pounds, increase stamina, ward of viral illnesses, reduce health risks, manage chronic conditions, strengthen the heart, keep arteries clear, boost mood, increase/sustain independence in old age, and prolong life (Mayo Clinic, 2012). Though these recommendations have positive impact for young and old, little is stated about what level of exercise is needed to increase one’s mental health, such as learning and memory. In addition, the recommendations add ambiguity by stating that activity performed on at least three days a week can produce health benefits (US Department of Health and Human Services, 2012), and that some physical activity is
better than none (Center for Disease Control and Prevention, 2011). No studies have systematically investigated various intensity levels or duration with regard to their effects on memory performance, nor have many studies investigated minimum levels of intensity or durations needed to affect memory performance. In addition, few to no studies have investigated whether intermittent exercising, such as every other day exercising, will have positive effects upon the brain and memory. Although the current exercise recommendations for humans are largely based on human research, findings from animal models can be informative. Continued research utilizing animal models allows the rigorous experimental control required to address questions concerning minimum duration or intensity levels needed to achieve physical and mental health improvements.

**Neurotrophic Factors**

**BDNF:** Brain derived neurotrophic factor (BDNF) is part of the neurotrophin family, and is indicated in the role of neuronal survival, maintenance, and growth (Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000). In addition, BDNF is indicated in neuroprotection and long term potentiation (Radecki, Brown, Martinez, & Teyler, 2005). Radecki et al. (2005) investigated whether BDNF could attenuate the effects of chronic immobilization stress on spatial memory and long term potentiation (LTP). Chronic stress is indicated in increasing levels of adrenal glucocorticoids, a stress hormone, which can result in decreased cognitive functioning (Radecki et al., 2005). The hippocampus is vulnerable to stress-induced damage. Thus, any organism, whether young or old, that experiences chronic stress may have spatial memory impacted. In order to measure the effects of chronic stress on animals, standard behavioral measures may be used. In addition, neurophysiological measures, such as LTP, may be used to assess cellular mechanisms of
neuroplasticity presumed to be involved in memory processes. Long term potentiation is a lasting enhancement in signal transmission at synapses due to rapid repeated electrical stimulation. LTP is most readily demonstrated in neural circuits involved in memory, such as the hippocampus. In Radecki et al.’s (2005) study, male Long-Evan rats were randomly assigned to stress treatment with BDNF infusion, or five control groups: stress treatment with saline infusion, stress treatment without saline infusion, nonstressed without infusion, and nonstressed with BDNF infusion. Prior to stress training, rats were anesthetized and cannulas were stereotaxically implanted in the left hippocampus. Minipumps were filled with either phosphate-buffered saline (PBS) or human recombinant BDNF. During stress treatment, animals were completely immobilized in plastic rodent restrainer for 2 h per day for seven days. After stress treatment, eight animals were decapitated and bilateral hippocampi were removed to study LTP. The remaining twelve animals underwent MWM training. During acquisition, each rat received two blocks of four 60 s trials per day for four consecutive days. Seven days after the fourth day was a probe trial. The authors reported the stress+saline group performed significantly worse than the unstressed+saline group in memory performance. Also, unstressed+BDNF performed at the same level as unstressed+saline, indicating that BDNF did not have an effect on spatial memory in unstressed animals. In contrast, the stress+BDNF group performed significantly better than the stress+saline group in the acquisition phase and probe trial, thus indicating that BDNF protected against stress-induced impairments. For LTP, authors reported that BDNF protected against LTP deficits since evidence for LTP in the brains of the stress+BDNF group was significantly stronger than in the stress+saline group.
Research also indicates that exercise is one of several ways in which BDNF is increased within the body (Griffin, Bechara, Birch, & Kelly, 2009). Griffin et al. (2009) investigated the effects of a 1 week exercise regimen on BDNF expression in the dentate gyrus. Male Wistar rats were subjected to treadmill exercise for seven consecutive days, 1 h per day, fluctuating between 10 – 15 m/min. Following the behavioral tests discussed previously (in “Forced Exercise” section), rats were decapitated and dentate gyrii were dissected free. Tissues were analyzed using a BDNF ELISA. Results indicate significant increase in BDNF concentration in dentate gyrus of exercise rats when compared to controls. This effect was also expressed in the hippocampus and perihinal cortex. In addition, BDNF increased performance on behavioral tasks. Rats that received a BDNF infusion performed significantly greater in the object substitution task.

GDNF: Glial cell line-derived neurotrophic factor (GDNF) is a recently discovered neurotrophic factor which has been indicated in supporting dopaminergic neurons of the central nervous system (McCullough, Peplinski, Kinnel, & Spitsbergen, 2011). Dopaminergic neurons are primarily located in the substantia nigra and ventral tegmental area (VTA) in the midbrain and within the hypothalamus. The midbrain VTA dopaminergic neurons project their axons to the prefrontal cortex. Though GDNF is more closely associated with skeletal muscle, the link between GDNF and dopaminergic neurons has led to the question of whether GDNF facilitates memory, such as spatial memory. This link is further solidified since dopaminergic neurons are indicated in contributing to cognition and learning and memory.

Thus far, few studies have investigated the interaction of GDNF with spatial memory. Pertusa et al. (2008) investigated the role of GDNF in spatial memory. In this
study thirty 22-month-old Fisher 344 rats underwent MWM training. Spatial training began with pre-training, which consisted of eight trials over two days, with the platform above the water surface. During the acquisition phase, the platform was placed below the water surface. Rats received four 90 s trials per day for 12 days. Immediately following the acquisition phase was the probe trial. The probe trial was used to split rats into impaired and unimpaired groups. Impaired rats were defined as those who spent time no different from chance performance in the right quadrant (correct quadrant). Those who spent time greater than chance in the right quadrant were labeled as unimpaired, thus indicating learning occurred during the 12 days. Two weeks after the probe trial, impaired rats were divided into two surgery groups. All rats received bilateral infusion into the CA1 of the hippocampus. One group received one microliter of the control substance, green fluorescent protein (GFP), while the other received one microliter of GDNF. Two weeks after recovery, rats received four trials per day with a probe trial on the fourth day. This procedure continued until 48 trials were conducted. The authors reported an effect of GDNF on spatial memory performance within the MWM. Specifically, escape latencies for the GDNF group were significantly shorter than the GFP group. In addition, escape latency for the GDNF group were comparable to the unimpaired group during the original, pre-surgery, MWM training.

Research also indicates that exercise influences GDNF levels. Currently, no published studies have examined the effects of exercise on GDNF within the brain; however, there are articles indicating that exercise can increase GDNF within the body. In a study by McCullough et al. (2011), 17-week-old Fisher 344 rats were used in in vivo exercise studies. Rats received forced exercise five consecutive days at 10 m/min for 45
min for a total of 2 weeks. After, the Extensor Digitorum Longus (EDL) and Soleus (SOL) hind limb was removed and processed using an ELISA. The authors reported that GDNF protein content significantly decreased in the EDL, whereas GDNF protein content significantly increased in the SOL. The authors suggest that muscle fiber may have impacted the results of GDNF protein content within the muscles. Specifically, a low-intensity exercise regimen may not have been enough to activate fast-twitch fibers, which primarily compose the EDL muscle (2011). Overall, these results suggest that exercise may have an impact on GDNF.

**BDNF and GDNF Summary**

Research indicates that both BDNF and GDNF may play essential roles in the development and maintenance of memory (Radecki et al., 2005; Pertusa et al., 2008). More importantly, the effects of both neurotrophic factors may be enhanced by exercise (Griffin et al., 2009; McCullough et al., 2011). Results from these studies contribute to the use of exercise as a non-pharmacological therapy, but also add additional information to possible mechanism of action for the effects of exercise, as well as increase the range of research areas in which exercise may benefit (e.g. neurodegenerative diseases).

**RESEARCH OBJECTIVES**

At the present time, relatively few studies are published regarding d-galactose as an animal model of aging, specifically with rats, nor are many studies published regarding exercise and d-galactose (Chae & Kim, 2009; Li, Ding, Marshall, Gao, Hu, & Xiao, 2011). The primary goal of this study was to investigate the effects of moderate intermittent exercise on the acquisition of a spatial memory task in d-galactose treated adult rats. A secondary objective was to investigate the effect of moderate intermittent
exercise on levels of BDNF and GDNF within two regions of the brain, hippocampus and prefrontal cortex.

**EXPERIMENTAL METHODS**

*Subjects*

Forty-eight male Sprague-Dawley rats (Charles River Laboratories, Portage, MI), approximately 6–8 months old at the beginning of the study, were used as subjects. All rats were singly housed in polycarbonate cages in a colony maintained on a 12:12 hour light/dark cycle (lights on 6am – 6pm) and constant temperature and humidity. Food and water was available *ad libitum* in home cages during the exercise portion of the study. Food was restricted during the radial arm maze procedure for approximately 14 h per day, with food availability beginning at approximately 10 p.m. and ending at approximately 8 a.m. All experimental procedures were conducted during the dark phase, under red light illumination.

*d-Galactose Treatment*

Rats were randomly assigned to a chemical senescence group or control group. Twenty-four rats were administered intraperitoneal (I.P.) injections of 100 mg/kg d-galactose (Amresco, Solon, Ohio) prepared in 0.9% saline and 24 rats were administered 0.9% saline injections once a day for eight weeks. This treatment regimen was based on previously published research (Fu, Wang, Li, Sun & Lu, 2010).

*Exercise Training*

During the same eight week period that the animals received daily d-galactose or saline injections, half of the animals in each group were randomly assigned to undergo exercise training three times a week on alternating days (e.g., M, W, F or T, R, S). The
remaining animals in each group were exposed to the apparatus for the same amount of time, but the running wheels were held stationary. Animals receiving exercise training were placed in individual running wheels (Lafayette Instruments, Lafayette, IN, USA) programmed to rotate at a speed of 13 m/min for 30 minutes (Chae & Kim, 2009). The rats in the non-exercise subgroups were placed inside the individual forced running wheels for 30 minutes, but not exercised.

**Radial Arm Maze Procedures**

Immediately following the eight week d-galactose treatment and exercise training period, all rats were trained to navigate an eight arm radial arm maze (108.5cm x 108.5cm) (Veena, Srikumar, Mahati, Bhagya, Raju, & Shankaranarayana Reo, 2009). Extra- and intra-maze cues were used during this procedure. Extra-maze cues consisted of textured and non-textured room walls, counter with sink, and a table. Intra-maze cues consisted of four shapes (circle, rectangle, triangle, and star; 12.7cm x 12.7cm) placed at cardinal points (North, East, South, and West respectively) 27.94 cm above the RAM.

The radial arm maze training consisted of two phases. The habituation phase began the day after the last injection day to familiarize the rats with the apparatus. All rats were subjected to two sessions on consecutive days. During the first day of habituation, each arm contained three Froot Loops® (beginning, middle, and end of arm). On the second day, each arm contained only two Froot Loops® (middle and end of arm). Each rat had 10 min to explore the maze during habituation.

Following habituation, a win-shift procedure was employed to assess acquisition over a 19 day period. This phase consisted of two five minute trials per day with an inter-trial interval of one hour. During trial one, only four arms (e.g. arm 2, 3, 6, and 8) were
baited and the four unbaited arms (e.g. arm 1, 4, 5, and 7) were blocked with a clear Plexiglas block. During trial two, all eight arms were open and the previously blocked arms were baited. All baited and blocked arms remained the same for each rat during this phase. Dependent measures consisted of percent correct arm choice, repeated arm entries, working memory errors, reference memory errors, time to first arm entry, time to complete task, and days to meet criterion. Correct arm choice was defined as the rat entering a baited arm and eating the Fruit Loop. Repeated arm entries were defined as entering an arm that was previously entered within that trial. Working memory errors were defined as re-entry into a baited arm where the bait was already retrieved. A reference memory error was counted as any entry into an un-baited arm during trial 2. Days to meet criterion was defined as the rat reaching 80% criteria (at least four correct arm entries out of five) for three consecutive days.

**Tissue Preparation**

The day following completion of the RAM procedure, animals were injected with sodium pentobarbital and decapitated. Immediately after decapitation, the hippocampus and prefrontal cortex were rapidly dissected over ice. Tissues were homogenized in a sample processing buffer (0.55 M NaCl, 0.02 M NaH$_2$PO$_4$, 0.08 M Na$_2$HPO$_4$, 2 mM EDTA, 0.1 mM benzethonium chloride, 2 mM benzamidine, 20 KIU/ml aprotinin, 0.5% BSA, and 0.05% Tween-20) in a 1:20 dilution and centrifuged for 30 min at 14,000 x g at 4 ºC. Supernatants were removed and stored at -80ºC for later analysis by enzyme-linked immunosorbent assays (ELISA for both BDNF (Griffin et al., 2009) and GDNF (McCullough et al., 2011).
**BDNF:** The concentrations of BDNF in supernatants were quantified by ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. Briefly, 96-well plates (MaxiSorp; NUNC) were coated overnight at 4 °C with monoclonal anti-BDNF antibody (100 μl per well) diluted 1:1000 in carbonate coating buffer (0.025M sodium bicarbonate, 0.025M sodium carbonate, pH adjusted to 7.2). Plates were then washed three times in TBST (20mM Tris-HCl, 150mM NaCl, 0.05% Tween 20) and blocked with block buffer (200 μl per well; block and sample 1x buffer) for 1 h at room temperature. Plates were then washed once in TBST. Next, plates were incubated with tissue supernatant and serially-diluted BDNF standards (range 0 – 1000 pg/ml) for 2 h at room temperature. Plates were then washed in buffer and incubated with secondary antibody anti-human BDNF pAb (1:500 dilution; 100 μl per well) for 2 h at room temperature. Plates were then washed again. Anti-IgY horseradish-peroxidase conjugated in blocking sample 1x buffer (1: 200 dilution; 100 μl per well) was added for 1 h at room temperature. Plates were washed and exposure with the color developer of TMB 1 solution was added for 10 min at room temperature. This reaction was stopped with 1N HCL. Absorbance was measured at 450 nm using a 96-well plate reader (Labsystems, Multiskan RC) within 30 min of stopping the reaction. Concentration of BDNF in samples was calculated by extrapolation from the standard curve, and results will be expressed as BDNF/mg tissue weight.

**GDNF:** GDNF protein content was determined using an ELISA (R&D Systems, Minneapolis, MN, USA). Briefly, 96-well plates were incubated overnight at 4 °C using a monoclonal antibody raised against GDNF (100 μl per well) diluted 1:1000 in carbonate coating buffer (0.025M sodium bicarbonate, 0.025M sodium carbonate, pH adjusted to
8.2). Plates were incubated for 1 h in block and sample 1x buffer (100μl per well). Plates were then emptied and tissue supernatant or GDNF standards (range 0 – 1000 pg/ml) were added to each well (200μl per well). These plates were incubated at room temperature for 6 h. Wells were then washed five times with TBST (20mM Tris-Hcl, 150mM NaCl, 0.05% Tween 20). After, anti-human GDNF pAb antibody (1:500 dilution; 100μl per well) was added and incubated overnight at 4°C. Plates were washed once and anti-chicken IgY horseradish-peroxidase conjugated in block and sample 5x buffer was added for 2 h at room temperature. Plates were then washed five times with TBST. TMB one solution was then added according to manufacturer’s specifications (100μl per well). The reaction was stopped using IN HCL and absorbance was measured at 450 nm. Concentration of GDNF in samples was calculated by extrapolation from the standard curve, and results were expressed as GDNF/mg tissue weight.

Data Analysis

All statistical analyses were conducted on SPSS (version 17) software (IBM Armonk, New York, USA), and graphs were created using Prism GraphPad (version 4.0) software (San Diego, CA, USA). All measures were analyzed either using a standard or repeated measures two-way ANOVA (exercise x drug treatment), a simple t-test, or a paired-sample t-test. Inter-observer agreement (IOA) was also calculated for all behavioral measures. IOA for two independent viewers was calculated as follows: subtract number of differing recordable events from total recordable events, divide by total recordable events, and multiply by 100 for IOA percentage. Body weight was calculated for eleven weeks (eight exercise weeks, three RAM weeks). For each week, an average for body weight was calculated. A combined average for those eleven weeks was
calculated and then used to estimate each rat’s body weight at the end of the eleven weeks (estimated body weight). In addition, the 85% body weight, normal body weight percentage during food restriction, was calculated for each rat at the end of the eleventh week.

RESULTS

IOA

Three independent observers reviewed the recorded video files of RAM trials and coded each rat’s performance. All three observers scored 100% of the videos. Interobserver agreement between two of the independent observers was 87.99% (range: 85.88% - 90.18%).

Body Weight

Body weight was calculated to ensure that the food restriction procedure did not significantly decrease body weight. Figure 1 depicts rats’ estimated body weight, 85% body weight, and actual body weight at the end of the eleventh week.

Figure 1. Mean (± S.E.M.) end body weight, estimated body weight, and 85% weight at the end of the eleventh week (Saline-EX: N = 12; Saline-NON: N = 12; Dgal-EX: N = 12; Dgal-NON: N = 12)
Paired-sample $t$-test indicated no statistical difference between estimated weight and the actual end weight ($t(47) = -0.54, p = 0.592$). However, there was a significant difference between actual end weight and 85% weight ($t(47) = 52.66, p < 0.001$).

**RAM Behavioral Measures**

Figure 2 depicts the average number of sessions to meet maze acquisition criteria. Three measures of maze acquisition were determined and compared among treatment groups: 1) a minimum of 80% correct arm entries in trial 1 for three consecutive days; 2)
a minimum of 80% correct arm entries in trial 2 for three consecutive days; and 3) a minimum of 80% correct arm entries in both trial 1 and 2 for three consecutive days. A two way ANOVA on the number of sessions to meet criterion performance in trial 1 showed no significant effects of d-galactose treatment or exercise. There was a significant effect of d-galactose treatment on trial 2 acquisition ($F(1,16) = 4.908, p < 0.05$), and both trial 1 and 2 acquisition ($F(1,15) = 5.462, p < 0.05$), but no significant effect of exercise on either of those measure of acquisition. Moreover, fewer animals in the d-galactose treatment groups met the minimum of 80% correct arm entries on three consecutive days compared to saline controls. Figure 3 depicts percentage of animals in each of treatment groups that met the criteria of minimum of 80% correct arm entries for three consecutive sessions for trial 1, trial 2, and both trial 1 and trial 2. While the

![Figure 3: Depicts percentage of animals to meet criterion for trial 1, trial 2, and trial 1 and 2.](image-url)
majority of the animals in all treatment groups met this criterion for trial 1, substantially fewer d-galactose treated animals met this criterion for trial 2 and trial 1 and 2. It is especially notable that only one animal in the d-galactose non-exercise group met the most stringent criterion.

For all other RAM behavioral measures, there was only a significant effect of test day, indicating that repeated exposure to the RAM increased performance in this spatial memory task. There were no significant effects of d-galactose treatment or exercise on any other measure of maze performance. Graphs of all dependent measures are displayed in Appendices section (Appendix A – E).

*Brain to Body Weight Ratio*

Figure 4 depicts brain to body weight ratio for all treatment groups. There was a trend towards exercise regimen having an effect on brain to body weight ratio, $F(1,47) =
3.163, $p = 0.082$. There are several indications for this trend. One of the main issues is that forced moderate exercise may be stressful to the organism (Yuede et al., 2009).

**BDNF and GDNF**

Figure 5 illustrates levels of BDNF and GDNF per mg of tissue. There was no statistically significant effect of exercise or d-galactose treatment on levels of BDNF or GDNF in the hippocampus and prefrontal cortex.

![Figure 5](image)

Figure 5. Mean (± S.E.M.) levels of BDNF and GDNF protein level within the hippocampus and prefrontal cortex. ($N = 12$ for each group).

**DISCUSSION**

This study empirically tested whether moderate forced exercise three days a week improved acquisition of a spatial navigation task or enhanced levels of BDNF and GDNF within the hippocampus and prefrontal cortex of adult rats. There are several potential clinical implications to research findings on the effects on forced exercise. Firstly, exercise may be considered a potential non-pharmacotherapeutic strategy used in adult or aged populations. Exercise may improve performance on cognitive tasks (spatial...
navigation, memory tasks, etc), or be an adjunct therapy for individuals who have neurodegenerative diseases. In addition, exercise may help individuals live independent lifestyles, or increase their ability to live in assisted-living facilities. Secondly, exercise may help individuals counteract the effects of ROS that are experienced throughout the environment (excess food consumption, environmental toxins, etc). If exercise counteracts the effects of ROS, there is a possibility that age-related cognitive deficits (as well as the possibility of other aged-related illnesses) could also be attenuated by exercise. The results of the current study indicate that moderate forced exercise three days a week had minimal effects on the acquisition of a spatial memory task in either d-galactose or saline treated rats. Although statistically significant main effects were observed for d-galactose treatment on the number of sessions to criteria for trial 2, and trial 1 and 2 combined, there were no statistically significant effects on several performance measures, including both working and reference memory errors. Furthermore, although the exercise regimen produced a nonsignificant trend on whole brain weight, there were no statistically significant effects of exercise or d-galactose treatment on BDNF or GDNF.

Several explanations may be considered when reviewing the negative results of this study. The first is whether intermittent exercise can impact spatial memory, or whether more frequent exercise may be required to produce effects. As previously discussed, suggested recommendations provided by health care agencies suggest a minimum of 150 min of moderate intensity aerobic exercise. However, two organizations (Center for Disease Control and Prevention, 2011; US Department of Health and Human Services, 2012) state that activity performed at least three days a week, or “some is better
than none,” can produce health benefits. Unfortunately, few studies have empirically validated the effects of intermittent exercise on brain health. The current study attempted to do so. The results of this study indicate that moderate forced exercise three times a week for 30 min at 13 m/min does not improve radial arm maze acquisition or performance in rats. In consideration of these findings, it is possible that 1) the duration of the exercise regimen was insufficient to increase spatial memory abilities in the particular RAM task employed, 2) the intensity of 13 m/min was insufficient to increase spatial memory abilities, 3) an exercise regimen of three days a week is insufficient to produce increases in spatial memory abilities, 4) the RAM procedure selected was not sufficiently sensitive disruption by d-galactose or improvement by exercise, and 5) the exercise regimen employed in the current study produced other health benefits not measured.

In lieu of the third point noted above, the majority of forced exercise literature that reports increased spatial navigation abilities adheres to an exercise regimen in which animals are exercised five to seven consecutive days (Fordyce & Wehner, 1993; Uysal et al., 2005; O’Callaghan, Ohle, & Kelly, 2008; O’Callaghan, Griffin, & Kelly, 2009; Hoveida, Alaei, Oryan, Parivar, & Reisi, 2011). However, these articles only confirm the primary suggestion provided by health care providers. Currently, there appear to be no exercise studies published within the past two decades that investigated the secondary suggestion that exercise three days a week provides health benefits, whether physical or mental.

Another consideration is whether forced exercise may be detrimental to organisms. A current debate within the exercise literature is whether forced exercise or
voluntary exercise differentially affect brain and behavior. Most of the arguments against forced exercise claim that forced exercise is stressful (Yuede et al., 2009) and produces anxiety-like behaviors (Leasure & Jones, 2008). However, some claim that stress, and associated elevated stress hormones, may be the underlying reason why animals forced to exercise display improvements in memory and learning performance (Ang, Dawe, Wong, Mochhala, & Ng, 2006). Few studies have compared forced and voluntary exercise (Arida, Scorza, Silva, Scorza, & Cavalheiro; 2004; Leasure & Jones; 2008; Yuede et al., 2009; Tascano-Silva, Silva, Scorza, Bonvent, Cavalheiro, & Arida; 2010). To briefly summarize, both voluntary and forced exercise produce beneficial effects in memory and learning performance, increase hippocampal volume, and increase neurogenesis. Thus, forced exercise and voluntary exercise produce similar effects on brain and behavior.

This information provides conflicting data as to whether results for whole brain weight in the current study may have been due to an aversive effect of exercise, or for other reasons, such as inconsistent excision of brain tissue.

A third consideration is administration route and the amount of d-galactose administered. Only a few published studies have examined d-galactose effects in rats (Chen, Lang, Zuo, Yang, Wang, & Xia, 2006; Kong, Wang, Wang, Hu, Han, & Liu, 2006; Hua et al., 2007; Chen, Lang, Zuo, Yang, & Wang, 2008; Lei et al., 2008; Lei, Hua, Xiao, Ding, Han, & Hu, 2008; Chen, Zhong, Peng, Sun, & Kong, 2010; Li, Gong, Wu, Lu, & Shi, 2010). In these studies, subcutaneous injection of d-galactose (range: 60 – 500 mg/kg daily for 6, 8, or 16 weeks) is the favored route of administration, whereas IP administration was only used in two studies. The first study administered 60 mg/kg daily for 6 weeks (Lei et al., 2006), but the authors only investigated the effects of d-
galactose on astrocytes (see results in “D-Galactose’s effects on Learning and Memory” section). The second study administered 20 mg d-galactose in 2 ml of saline (Hua et al., 2007). The authors used a Y-maze and passive avoidance test to investigate the effects of d-galactose on learning and memory. The Y-maze consisted of three identical arms. A correct response was recorded if the rat moved directly into the secure arm of the Y-maze when it received a foot shock. The learning criterion was nine correct responses within ten continuous training trials. The passive avoidance task consisted of an acrylic box with a stainless steel floor, and a platform that was at the center of the box. Electrical shocks were delivered through the steel floor. Training began with the rat acclimating to the box for 3 min. Testing occurred immediately after the 3 min. Testing consisted of the rat being placed on the platform. Measures consisted of number of errors made, how many times a rat jumped off the platform after the first shock delivery, and step-down latency, time of staying on the platform, within a 5 min period. The results indicate that rats treated with d-galactose needed significantly more training trials when compared to controls. In addition, d-galactose treated rats made significantly more errors, as well as had shorter latencies to step-down, when compared to control rats. Overall, there is no conclusive data as to what specific IP dose, or range of doses, would affect spatial memory in a rat animal model. The results of the current study indicate that daily administration of d-galactose (100 mg/kg, IP) for eight consecutive weeks was insufficient to produce statistically significant learning deficits in a RAM procedure.

The last consideration is whether passive exposure to the exercise apparatus increased cognitive and spatial abilities. In a study conducted by O’Callaghan, Griffin, and Kelly (2009), middle-aged male Wistar rats were subjected to forced moderate
exercise on motorized treadmills. The exercise regimen consisted of two 30 min sessions at 16 m/min, with a 30 min break between sessions, seven days a week for eight months. Middle-aged control rats were placed also placed into stationary treadmills for the same amount of time as the experimental animals. At the end of the eight month period, LTP and MWM procedures were performed. In addition, a group of young rats and middle-aged sedentary rats were also subjected to the same experimental procedures. At the end of the eight months, the middle-aged groups were known as “aged”. The authors reported no significant difference in escape latency among young, aged treadmill control, and aged exercised rats. However, there was a significant difference between young, aged control, aged exercise, and aged sedentary rats. These results were also similar when assessing LTP, nerve growth factor (NGF), and BDNF in the dentate gyrus among groups. The authors hypothesized that removal from the home cage, regular handling, and exposure constituted a form of environmental enrichment that increased spatial memory performance, LTP, and expression of NGF and BDNF within the dentate gyrus. In lieu of these results, the lack of significant findings in the current study may have been due to effects of repeated handling and exposure to the exercise apparatus in the non-exercise controls. However, one important difference between O’Callaghan’s (2009) and this current study is the difference between the duration of the exercise regimen. O’Callaghan’s (2009) regimen lasted eight months, while this study’s regimen lasted eight weeks. It is unclear whether eight weeks of repeated handling and exposure to a stationary running wheel is sufficient to produce an effect. Further studies including a sedentary control group are required to address this question.
Furthermore, handling and exposure to the RAM apparatus may have also skewed results regarding any potential effects of d-galactose on BDNF and GDNF levels in the hippocampus and prefrontal cortex. It is possible that BDNF or GDNF levels could be altered by d-galactose or exercise, but any effects of these treatments were attenuated by exposure to the RAM task. A separate group of animals without RAM acquisition training would be required to address this question.

**Future Research Extensions**

Variations of the current study could be considered for extension in future research. One of the first augmentations would be to vary the intensity (m/min), duration of time spent within the individual exercise wheels, or number of weeks/months adhering to the exercise regimen. This would provide more information on whether exercise three days a week would provide mental health benefits. This would also clarify the ambiguity in the health services statement of three days a week of exercise provides health benefits.

A second change would be to increase the number of days exercised, as well vary the amount of consecutive exercise days. For example, if the number of exercise days were increased to four, one could investigate several variations. Some of these variations could include: 1) one exercise day, two non-exercise days, three exercise days; 2) two exercise days, two non-exercise days; two exercise days; one non-exercise day. This variation would systematically expand forced exercise literature. The results provided by these studies could be extrapolated to the human population. The information could then be used by health departments to recommend various exercise strategies that would only benefit mental health, physical health, and weight loss.
A third variation would be to study a range of IP doses of d-galactose. Thus far, only two studies have investigated IP doses, with one specifically investigating the effects of d-galactose on learning and memory. The addition of this type of study would provide the following information: 1) a range of doses that would produce a decrease in spatial memory ability, 2) whether d-galactose has a dose-dependent effect on spatial memory performance, and 3) the most efficacious dose to produce deterioration in spatial memory abilities.

A fourth and perhaps most important variation would be to add a cage sedentary group to the current study. This group would consist of rats that were not exposed to the exercise apparatus, but also another group that was not exposed to the exercise apparatus or the RAM. The addition of these two sedentary groups would provide more information about the following aspects of this study: 1) does d-galactose treatment decrease spatial memory performance, 2) can exercise attenuate the effects of d-galactose, 3) can exercise increase levels of BDNF and GDNF within the hippocampus and prefrontal cortex, and 4) can exercise three days a week increase spatial memory performance in a RAM.

Overall, if exercise is to be considered an alternative to pharmacotherapy for middle-aged or aged individuals, or even an adjunct therapy for those with neurodegenerative diseases, further research on varying intensities, durations, and schedules of exercise must be investigated. In as much as the results from experimental studies in animal models can be extrapolated to human health conditions, they may potentially provide essential information regarding best practices for health improvement and may be useful in determining the recommendations offered by health organizations.
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with ageing is counteracted by maze learning earlier in life but not by physical


Appendix A

RAM Behavioral Measure – Latency to First Arm

Figure 6. Mean (± S.E.M.) latency to enter the first arm for trial 1 and trial 2 once the rat was placed within the RAM. There was a significant effect of day for both trial 1, $F(18,37) = 2.538, p < 0.001$, and trial 2, $F(18,41) = 3.281, p < 0.0001$, thus indicating that continual exposure to the apparatus increased performance.
Appendix B

RAM Behavioral Measure – Time to Complete Maze

Figure 7. Mean (± S.E.M.) total amount of time rats took to complete the maze task. There was a significant effect of day for both trial 1, $F(18,28) = 26.084, p < 0.0001$, and trial 2, $F(18,41) = 25.272, p < 0.0001$, thus indicating that repeated exposure to the apparatus decreased total time to complete the RAM procedure.
Appendix C

RAM Behavioral Measure – Working Memory Errors

Figure 8. Mean(± S.E.M.) total amount of working memory errors for trial 1 and 2. There was a significant effect of day for trial 1, $F(18,35) = 5.645, p < 0.0001$, and trial 2, $F(18,40) = 3.201, p < 0.0001$, thus indicating that repeated exposure to the apparatus decreased working memory errors.
Appendix D

RAM Behavioral Measure – Reference Memory Errors

Figure 9. Mean (± S.E.M.) reference memory errors incurred in the RAM during trial 2. There was a significant effect of day, $F(18,40) = 9.997, p < 0.0001$, thus indicating that repeated exposure to the apparatus decreased amount of reference errors.
Appendix E

RAM Behavioral Measure – Total Repeat Errors

Figure 10. Mean (± S.E.M.) amount of repeated arm entries. There was a significant effect of day for trial 1, $F(18,35) = 17.466, p < 0.0001$, and trial 2, $F(18,40) = 10.173, p < 0.0001$, thus indicating that repeated exposure to the apparatus decreased the amount of repeated arm entries made by rats.
Appendix F

Approval Letter from Institutional Animal Care and Use Committee

Date: March 9, 2011
To: Lisa Baker, Principal Investigator
From: Robert Eversole, Chair
Re: IACUC Protocol No. 11-02-01

Your protocol titled “Effects of Moderate Forced Exercise on Spatial Memory in Rats Senesced with D-Galactose” has been reviewed by the Institutional Animal Care and Use Committee. Before final approval can be given the following concerns should be addressed and revisions submitted for review by the IACUC chair:

1. Please correct the typographic errors:
   - Date submitted to funding agency: 03-15-2011
   - Date of Search: 01-13-2011

Please submit a cover letter describing the above changes and one copy of the revised protocol to the IACUC, 251W Walwood Hall, mail stop 5456. Remember to include the IACUC project number (above).

If you have any questions, please call the research compliance coordinator at 387-8293.