

The effects of voluntary exposure to an enriched environment on olfactory function in Alzheimer's Bace1 mice models

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Allegheny College
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**A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College**

*I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole.*

Pledge

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ABSTRACT

Alzheimer's disease (AD) is a type of dementia identified by deterioration in memory, cognitive abilities, language and motor skills. It occurs when there is an accumulation of amyloid plaques in the brain, accompanied by neurofibrillary tangles. The accumulation causes neuronal death, which severely impacts cognition. Drug therapies may aid in treating the symptoms of Alzheimer's, but do not prevent or cure AD. Exercise and cognitive rehabilitation have been shown to improve cognitive functioning in AD patients. Providing an enriched environment to promote physical activity and stimulate cognitive capacities may be an effective way to manage AD. Because decline in olfactory function is often correlated with the progression of AD, I tested the hypothesis that environmental enrichment and exercise would slow the deterioration of olfactory performance in AD model mice. Twelve Alzheimer's Bace1^{tm1Pcw} mice models, originating from the Jackson Labs and bred in the Allegheny College Biology department mice model colony, were used for this study. Three different age groups of four mice each were used: 7 weeks, 4 months, and ~5 months old. Two mice from each age group were assigned to treatment and control groups. Treatment Bace1 mice were exposed to enriched environment activities for 3+ hours, three times a week for a six-week period. Control Bace1 mice were kept in normal cages during the entirety of the experiment. I tested olfactory function via "the hidden cookie test" at pre-treatment, during treatment, and multiple times post-treatment. Results show that deterioration of olfactory function occurred with all mice, but the treatment group showed lessened effects while the control group worsened over time. The age at which physical enrichment was initiated had no significant effect on olfactory function. My results suggest that environmental enrichment and physical activity has the potential to slow the progression of AD symptoms.

INTRODUCTION

Alzheimer's disease (AD) is the sixth leading cause of death in the United States, and the most common form of dementia (Droes et al. 2011, Alzheimer's Basics 2012). It is a neurological disorder that affects cognitive and intellectual capacities, in addition to the ability to form and retain memories. It is characterized by the formation of amyloid plaques and neurofibrillary tangles in the brain. Plaques are protein deposits that block spaces between neurons, and the tangles build up within the nerve cells. As we age, it is common to find minimal deposits of plaques and tangles, but Alzheimer's patients have an abnormally large amount. The exact mechanism of how these proteins block communication and generate memory loss is unknown (Alzheimer's Basics 2012).

The amyloid cascade hypothesis is widely accepted by the scientific community as the general explanation of AD's deterioration of the brain (Matsumoto et al. 2013). It occurs in four steps. First there is accumulation in the brain of amyloid plaques composed of beta proteins. Then, tau phosphorylation occurs as tau proteins become defective and do not stabilize the nerve fibers of the brain. Neurofibrillary tangles result as tau proteins begin to clump. As a consequence, neurons degenerate and connections are lost (Matsumoto et al. 2013).

One of the greatest risk factors for developing Alzheimer's is old age (Hargreaves 2012). One in every three seniors will die with Alzheimer's or another form of dementia (Alzheimer's Basics 2012). Alzheimer's is a progressive disease, which means that it gradually worsens over time. The early stages show a moderate decline in memory, while the later stages show a severe impact on cognitive ability and daily functioning. The plaques and tangles cause deterioration in the brain, and affected individuals experience difficulties with language, motor skills, attention, and recognition (Droes et al. 2011). All over the world the elderly population is exponentially

increasing. Numbers are expected to approach two billion by 2050. Effective treatments for AD are particularly timely, especially because of the aging baby boomer generation and the strain it will place on our healthcare system (Hargreaves 2012).

There are approximately 5.4 million people suffering from Alzheimer's disease in the United States, and this number is predicted to escalate dramatically in the next few decades. As of now, there is no cure for AD and no way to stop or restrain the disease (Hargreaves 2012). Because Alzheimer's disease can only be diagnosed post-mortem, many cases are unknown. Current treatments include drugs that only attend to symptoms of AD, not cure them. Physical exercise and cognitive rehabilitation are possible alternatives and may be used in combination with drug therapy (Andrade and Radhakrishnan 2009). Many factors contribute to the complexity of Alzheimer's disease, which has inhibited our understanding of ways to treat it. Alzheimer's disease not only affects those that are diagnosed, but the families and caregivers of each patient. The time to conquer the medical and societal aspects of Alzheimer's disease is now more than ever (Hargreaves 2012).

Pharmacological Treatments for Alzheimer's Disease

There is not a single completely effective drug treatment for Alzheimer's disease (Yisnopoulou and Papageorgiou 2013). The drugs developed for the treatment of Alzheimer's symptoms can slow brain deterioration and possibly aid in neuron signaling (Droes et al. 2011). Drugs used to treat AD include donepezil, rivastigmine, galantamine, and memantine, which are symptomatic and do not counteract the continual formation of plaques and tangles. Donepezil and rivastigmine are cholinesterase inhibitors. Alzheimer's patients have lower levels of ACh, so these drugs target the enzyme acetylcholinesterase to increase ACh. This is thought to enhance cognitive function (Adams and Page 2002). These drugs are used only during the early stages of

Alzheimer's. Other forms of treatment that have been tested but not proven effective or beneficial include an Alzheimer's vaccine (target tau proteins), statins (reduce triglycerides and cholesterol) and estrogen supplements (Helmuth 2002).

A recent DNA vaccination developed in mice models, called YM3711, shows that the vaccine greatly reduced amyloid beta plaques (Matsumoto et al. 2013). The vaccine does this by inducing an increase in the antibodies that target and attack amyloid beta plaques. It is not clear whether this method of reducing amyloid plaques will actually prohibit or halt further development of the disease. The most recent immunotherapy for AD was terminated in phase II of the trial due to 18% of the participants becoming ill with meningoencephalitis, an inflammatory infection of the brain (Matsumoto et al. 2013). No further success has been granted with this particular therapy.

Although these drugs show some benefit, they are only treating the symptoms and do not work for all patients (Adams and Page 2000). Other pharmacological interventions have yet to pass clinical trials and gain approval for the market (Yiannopoulou and Papageorgiou 2013). The complexity of AD makes it hard to target prevention strategies with various medications. While researchers develop a better understanding of the disease, it may be wise to invest ourselves in the management of AD symptoms with immediate and available alternatives (Helmuth 2002).

Exercise and Alzheimer's Disease

Adult hippocampal neurogenesis (formation of nervous tissue) has become apparent as a possible preventative measure for Alzheimer's disease. The hippocampus is crucial for learning and memory, both of which decline in neurodegenerative diseases (Yau et al. 2014). Physical exercise may boost cognitive performance by maintaining neurogenesis. Exercise increases

blood flow to the brain, angiogenesis, and brain plasticity. The exact mechanism between exercise and neurogenesis is still unknown, although we know humans obtain many long-term positive effects from engaging in regular physical exercise. Although it may not entirely prevent Alzheimer's from developing, exercise has the potential to delay its onset and offset some of the other debilitating symptoms (Yau et al. 2014).

Results of a recent study show a possible connection between AD and enriched physical environments when examining hippocampal neurogenesis in mice models (Madronal et al. 2010). Spatial learning, rotarod performance, and instrumental conditioning all improved after mice were exposed to physically enriched environments. Because neurogenesis decreases with age, physical activity had more pronounced effects in the younger mice (Madronal et al. 2010). A limitation of this study includes forced physical activity on the rotarod, which may have induced stress and anxiety, yielding less pronounced results. It would be interesting to study the effects of solely voluntary exercise, rather than exercise that is forced.

Olfactory Function and Alzheimer's Disease

Olfactory function may be a potential biomarker for Alzheimer's disease (Wesson et al. 2010). A side effect that has been seen in most individuals with Alzheimer's disease is a loss or impairment of olfaction. In mutated mice that over express amyloid plaque deposits, deposits first occurred in the olfactory bulb before any other location in the brain. This may be an explanation as to why Alzheimer's affects olfactory function (Wesson et al. 2010). The mice in this experiment were tested using an odor-cross habituation test where various chemicals were inserted in their home cages and observations recorded on the amount of time the mice spent sniffing and investigating the new smell (Wesson et al. 2010). The mutated Tg2576 mice showed impaired functioning in olfaction compared to the wild-type control mice. The earliest olfactory

dysfunction was recorded at mice aged 3 months old (Wesson et al. 2010). An extension to this study would be to introduce AD treatment methods at various life stages and test if olfactory function improves or stays the same instead of declining as the disease progresses.

The purpose of my study was to use olfactory function, assessed by the hidden cookie test, as a measure of the severity of Alzheimer's disease, while introducing enriched environments to AD model mice at 7 weeks, 4 months, and ~5 months of age. Previous studies have not examined the effects of voluntary physical exercise in combination with early vs. late intervention. Here, I provided an enriched environment for treatment mice to stimulate voluntary physical activity and to test its effects on the management of Alzheimer's disease. I also examined the effects of age and time of intervention on olfactory performance of subjects over time in regards to age and difference in time of intervention. Although mice at different ages may have differences in olfactory function and cognitive abilities at the start of treatment, my objective was to examine the change in olfactory function over time.

I hypothesized that the youngest age group receiving early intervention treatment would perform the hidden cookie test faster than the other two age groups. I also hypothesized that olfactory performance of mice in the treatment group would decrease over time, while olfactory performance of control group mice would stay the same or increase.

METHODS

Ethics Statement

This experiment was performed in accordance with the Animal Research Committee of the Allegheny College Biology Department. The proposal for this experiment was accepted in October 2015 and animals were monitored throughout experimentation.

Animals/Treatment Protocol

Twelve Bace1 mice were obtained from the Allegheny College biology department mice colony. The breeding pair of this specific colony originated from the Jackson Labs. The Bace1 mutants have increased levels of the Bace1 enzyme, which is needed to produce beta amyloid peptides. Beta amyloid peptides make up amyloid plaques, the characteristic condition of AD (Cole and Vassar 2007). The Bace1 mouse model is used to support research in neurobiology, and more specifically Alzheimer's disease. Compared to wild-type, these mice display impaired cognitive functioning (<https://www.jax.org/strain/004714>).

Mice were housed in twos after weaning and kept in those same pairs for experimentation due to the social nature of mice. All mice were kept under standard conditions, except during treatment and testing days. Four mice from each age group were randomly assigned to the control or enriched treatment groups, with two mice from each age group in the control group and two mice from each age group in the treatment group. In total, there were 6 control mice (two 7-week old mice born in September 2015, two 4-month old mice born in July 2015, and two 5-month old mice born in May 2015) and 6 treatment mice (two 7-week old mice, two 4-month old mice, and two 5-month old mice). The sexes of mice were not evenly distributed due to colony constraints. See Table 1.

Table 1: Mouse identifications and basic information for the 12 Bace1 mice used in this study

<u>Mouse ID</u>	<u>Cage #</u>	<u>Birth Date</u>	<u>Group ID</u>	<u>Gender</u>
1	1	09/10/2015	Control	Male
2	1	09/10/2015	Control	Male
3	2	09/10/2015	Treatment	Male
4	2	09/10/2015	Treatment	Male
5	3	07/06/2015	Treatment	Male
6	3	07/06/2015	Treatment	Male
7	4	07/13/2015	Control	Male
8	4	07/13/2015	Control	Male
9	5	05/07/2015	Control	Male
10	5	05/07/2015	Control	Male
11	6	05/25/2015	Treatment	Female
12	6	05/25/2015	Treatment	Female

This study lasted approximately 7 weeks. The last week of the experiment did not involve enrichment, and was used for the post-treatment hidden cookie test trials. Treatment mice received 6 weeks of enriched activity in the enrichment cage at 9 hours per week. Control mice did not receive enrichment and had normal activity in a regular sized cage. All other hours for the treatment group were spent in a normal cage.

Mice were handled four-five times per week for treatment, testing and cage cleaning. Control mice were handled on treatment days to minimize a handling effect. Treatment mice were moved to an enrichment cage for 3 hours, 3 times a week. The enrichment cage (about twice as large as a standard cage) contained a running wheel, chew toys, a playhouse, connected tubes, and novel play toys (Fig. 1). The regular cages were stocked solely with bedding, food, and water.



Figure 1: Image of enrichment cage.

Hidden Cookie Test

All mice were subject to the hidden cookie test. The hidden cookie test was performed pre-treatment, during enrichment treatment, and five times post-treatment. The hidden cookie test for this study was adapted from Yang and Crawley (2009). Nabisco Nutter Butter cookies were used for testing. Mice were put under food deprivation for each hidden cookie test. Pre-treatment food deprivation was 14 hours, during treatment food deprivation was 18 hours, and post-treatment food deprivation was 24 hours. The amount of time for food deprivation changed due to willingness of mice to participate in testing. Food was restored immediately after testing and mice were allowed to keep and eat the cookie once found.

The hidden cookie test took place in an empty enrichment cage with nothing but bedding and the hidden cookie. Mice were timed to see how long it took them to find the cookie hidden randomly in the cage. Mice were tested individually. The cut-off time for each subject was 15 minutes. A normal mouse without the *Bace1* mutation usually finds the cookie in less than a minute (Yang and Crawley 2009). The timer was stopped when the mouse picked the cookie up and began to eat (Yang and Crawley 2009). Times were recorded for all mice.

Statistics

Hidden cookie test data were analyzed with the statistical package JMP Pro 12.0. Tests of significance were performed with a generalized linear mixed model in which treatment group (two levels: enriched vs. control), time category (seven levels: pre-treatment, during treatment, and 5 post-treatment tests), the treatment x time interaction, and mouse birth months (three levels: May 2015, July 2015, and September 2015) were entered as fixed effect and mouse identity and cage number were used as random effects to control for shared housing and multiple measures from the same mouse. Time to complete the task (find the cookie) was log transformed to equalize variances.

RESULTS

Time to find the hidden cookie generally increased over time for all mice; however, treatment mice exposed to enrichment and voluntary physical activity showed dramatic reductions in time to find the cookie during post-treatment tests (Figure 2). The general linear mixed model showed significant effects of both time category and the treatment x time interaction (Table 2).

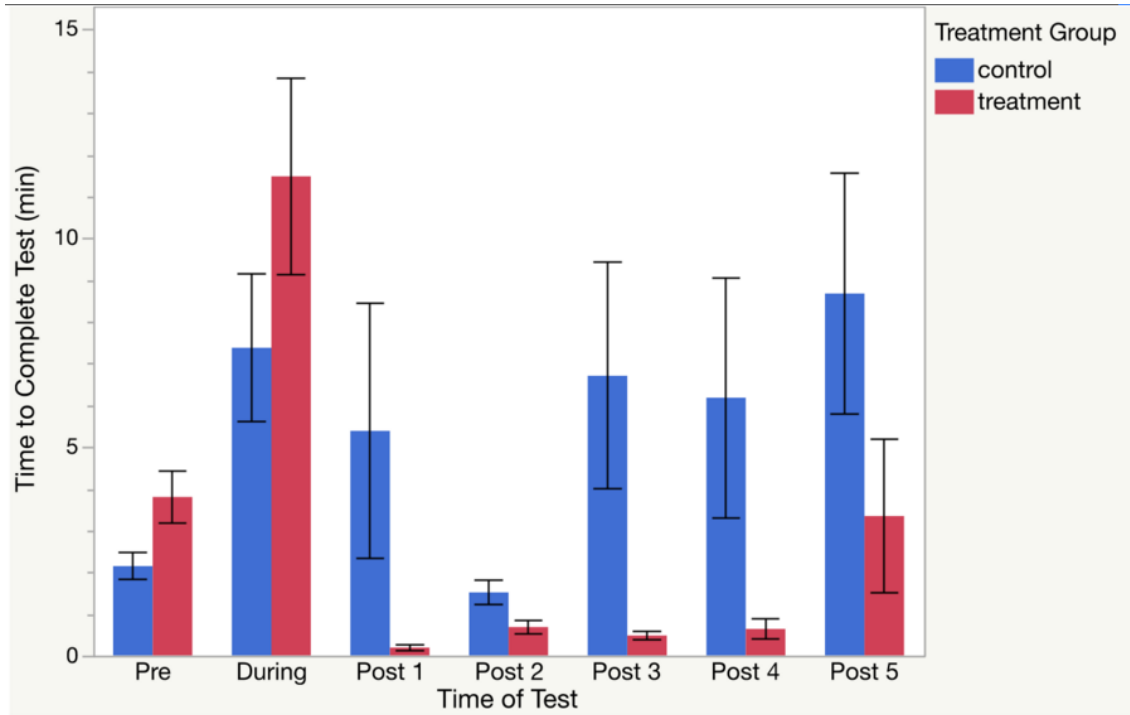


Figure 2. Time in minutes to complete the hidden cookie test over 7 week period in control and treatment groups. Error bars represent the standard error of the mean.

Mouse age category did not have a significant effect on performance on the hidden cookie test (Table 2). Within each age category, the mice in the treatment group generally performed better (faster) on the hidden cookie test post-treatment than did control mice (Fig. 3).

Table 2: Fixed effect tests for general linear mixed model of log-transformed times to find the hidden cookie. Mouse identity and cage number were included in the model as random effects.

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment Group	1	1	2	16.1557	0.0567
Time Category	6	6	60	8.0078	<.0001*
Treatment Group*Time Category	6	6	60	3.3479	0.0065*
Age Group	2	2	2	1.3033	0.4342

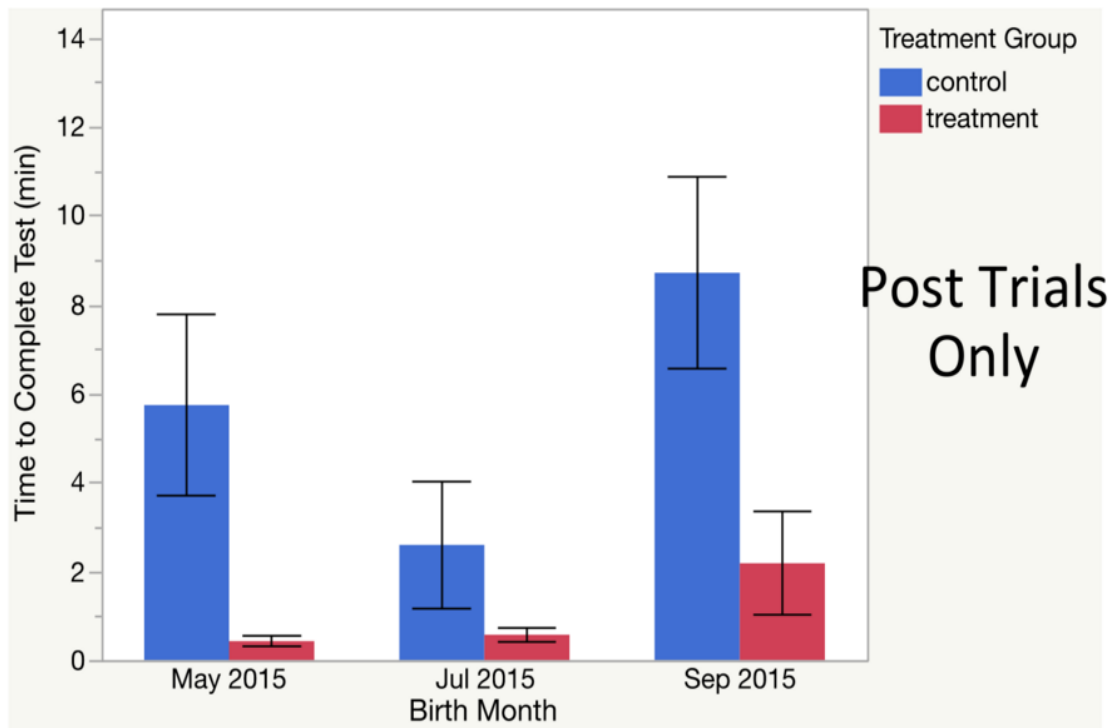


Figure 3. Relationship between age category (birth month) and time in minutes to complete the hidden cookie test in post-treatment trials only with control and treatment groups. Error bars represent standard error of the mean.

DISCUSSION

Effects of Voluntary Exercise and Age on Olfactory Performance

Overall, this study showed that voluntary exercise greatly improved olfactory performance in the hidden cookie test in the post-treatment trials (Fig. 2). I hypothesized that olfactory performance of mice in the treatment group would decrease over time, while olfactory performance of control group mice would stay the same or increase. Results show that although both groups declined, the treatment group declined at a lesser rate (Fig. 2). This comparison was best seen at the end of the 7-week period in the post-treatment trials. Perhaps increasing the

amount of exercise time during the week or prolonging the study in general would increase performance over time and in an even more significant way.

The effects of exercise seem to be delayed. In this study, there was no evidence of an exercise effect in the during-treatment period, when enriched mice were compared to the control mice (Fig. 2). Again this could be due to the length of the study or the amount of exercise the treatment mice received each week. It would be interesting to test varying amounts of exercise per week to find an optimal amount for enhancing olfactory performance. It was noted throughout the study, during enrichment observation periods, that the mice were most attracted to the running wheel over all other enrichment activities to choose from. It may be of interest to look at just the effects of the running wheel compared to the other enrichment activities (chew toys, large open area, etc.).

In addition, voluntary exercise seemed to be equally effective for all three age groups of mice (Table 2, Fig. 3.). I hypothesized that the youngest age group, with the earliest intervention of treatment, would receive the most benefit from enrichment and perform fastest on the hidden cookie test. The three age groups of mice used for this experiment were approximately two months apart. This may have not been a large enough gap to compare age groups with various stages of Alzheimer's. The Bace1 mice used in this study live to approximately two years old (<https://www.jax.org/strain/004714>). The original hypothesis may be supported if the three age groups included mice at weaning (6 weeks), 1 year, and 2 years old. All in all, these results suggest voluntary physical activity at any age could be valuable as a therapy for AD. It is possible that exercise could serve as a universal therapy for all AD patients while we await a cure for Alzheimer's disease.

Exercise Used to Reverse Unhealthy Brain Aging in Female C57 BL/6 mice, a common inbred strain of lab mice

Female C57 BL/6 mice, a common inbred strain of lab mice, aged approximately 1 year show that exercise decreases hippocampal astrocytes and myelin markers of aging and increases a marker for new blood vessels to form (Latimer et al. 2011). Exercise mice were caged with a running wheel while the sedentary mice were housed in regular cages for six weeks. This study shows a possible window at middle-age where exercise intervention could be used to improve brain function (Latimer et al. 2011). A sedentary lifestyle can exasperate the aging of the brain and exercise has the potential to reverse those effects (Latimer et al. 2011).

Although the study by Latimer et al. (2011) was not performed with AD model mice, it would be interesting to test if exercise intervention at middle age could delay or completely offset the disease. In this specific study, exercise decreased the unhealthiness of astrocytes. Astrocytes are a type of glial support cell in the central nervous system (Latimer et al. 2011). This provides insight into a connection between exercise, astrocytes, and healthy brain activity and further research could explore a possible relationship of astrocytes with Alzheimer's disease.

The Alzheimer's Pill

A recent article in Time Magazine highlights Dr. Frank Longo, chairman of the neurology and neurological sciences departments at Stanford University, who has developed a new drug for Alzheimer's called LM11A-31 or C31 (Park 2016). Most drugs that are currently on the market target the amyloid plaque build-up in the brain and work to break that down. Some drugs eliminate excess amyloid to help prevent the plaques from forming to begin with. Neither of these methods has aided patients' symptoms of Alzheimer's disease. Dr. Longo's drug works to protect neurons, our brain cells, and strengthen the connections between them (Park 2016).

All patients with Alzheimer's disease have amyloid plaque build-up in the brain; however, some people have amyloid in the brain but do not develop Alzheimer's disease. The key to diagnosing Alzheimer's is monitoring the growth of the amyloid (Park 2016). Tau proteins appear in the later stages, characterized by the neurofibrillary tangles, and causing the most severe losses of memory and cognitive ability. There is no "one drug solution" to treat the many facets of this disease (Park 2016).

C31 works to prevent neuron deterioration and reverse current amyloid damage in addition to increasing nerve-growth factors (Park 2016). This drug has been successful in mice; however, the tricky part is transitioning that success to humans. C31 would have to be combined with multiple other drugs – drugs yet to be made to combat Alzheimer's completely. The most effective way to treat Alzheimer's is early intervention. Every patient is unique, however, and present with Alzheimer's at different ages and in different ways. Imaging techniques to scan for amyloid build-up is thousands of dollars per patient per scan (Park 2016). The main issue at this time is reversing the damage of Alzheimer's with the millions of people that are already living with it. At this point in time, it has been impossible to reverse neuronal deterioration (Park 2016).

Plaque Clearance in Alzheimer's Mice

Seventy-five percent of treated Alzheimer's mice showed plaque clearance and improved performance on memory tasks via repeated ultrasound scanning treatments (SUS), which works to remove amyloid beta peptide in mouse brains (Leinega and Götz 2016). Twenty APP23 mice, showing over expression of beta amyloid peptide and plaque deposits, were tested against 10 non-transgenic (do not have an altered genome) littermates, where the SUS treated mice improved in long and short term memory tasks. SUS works by opening the blood brain barrier

and stimulating neuronal activity. The blood brain barrier is a filtering mechanism used by the brain's vascular system to carry oxygen and nutrients to the brain while simultaneously blocking unwanted substances. The SUS treated mice showed increased microglia activity, where the support cells of the brain helped takeaway plaque build-up (Leinega and Götz 2016).

This recent study shows another possible route to treat Alzheimer's, however limitations still remain. To start, the mice models used do not fully express the pathology of Alzheimer's disease exhibited in humans. Access to a mouse brain via SUS is much easier than finding an access point for humans (Leinega and Götz 2016). The ultrasound scanning treatments were performed on mice with severe plaque build-up so results are ambiguous toward early stages of Alzheimer's (Leinega and Götz 2016).

Alzheimer's disease in Developing Countries

Dementia is a disease found all over the world, affecting the aging population of all cultures and geographic locations (Kalaria et al. 2008). The first obstacle to conquering dementia worldwide is the absence of a standardized screening method. The Mini-Mental State Examination is commonly used in the United States as a screening tool; however, has yet to be translated in multiple languages and cannot be used with patients who are illiterate. Alzheimer's is the most common subtype of dementia found in developing countries (Kalaria et al. 2008).

Conditions surrounding poverty, which are more likely encountered in developing countries than developed countries, may lead to an increased risk of developing Alzheimer's disease. These poverty-stricken areas are likely to have no or little access to health care and the necessary means to aid with cognitive decline (Kalaria et al. 2008). Even if a drug were available that cured Alzheimer's disease, access to this drug in developing countries would not exist. Furthermore, a cure at this time does not exist and further research is needed to attain a cure. In

the meantime, awareness of Alzheimer's disease and the positive effects of exercise need to be promoted.

Limitations

Some limitations of this study include small sample size and an uneven distribution of males and females. Twelve mice were used in total; however there were only two mice per age group for treatment. Random effects of baseline individual differences could have affected results. There were no visual physical abnormalities or noticeable testing differences between the male and female mice; however sex-based differences do exist (Dubal et al. 2012). It may be beneficial to use a larger sample size, randomize mice at birth and have an even sex distribution to control for these limitations. Improving these limitations would allow results to become more applicable to managing AD in humans.

Future Studies

Further research should be done with prolonged enrichment and with early to late stages of Alzheimer's. Different amounts of enrichment per day and per week should be tested to find an optimal time of enrichment. It would also be beneficial to test the differences between males and females and beginning to transition this type of research from mice to humans. Enriched environments could serve as a potential intervention for individuals suffering from AD with no access to healthcare or living in developing countries where medicines used to manage AD are not available.

Conclusion

Our limited understanding and insufficient knowledge regarding the degenerative process of Alzheimer's disease has created the need for further research and innovative methods to cope with its symptoms. Medical and societal advances regarding Alzheimer's disease are vital toward

the health of our nation and the world. Alzheimer's disease affects patients, families, caregivers, and the current health care systems in place. "In the U.S. alone it is estimated that more than one trillion dollars will be needed annually to provide healthcare for AD patients, producing a societal public health economic burden that will fast become unsustainable," (Hargreaves 2012). Drugs and other pharmaceutical therapies have not proven successful, and although exercise alone does not cure Alzheimer's, it can be beneficial to patients awaiting a cure and to those who do not have access to available medications.

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