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Analyzing the Use of 5XFAD Model Mice in Determining Late-Stage Alzheimer's Disease Treatment

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ABSTRACT

Nationally, Alzheimer's Disease (AD) is growing in numbers across an elderly demographic. While treatments exist to mitigate degeneration, they usually are targeted towards early-stage Alzheimer's and tend to neglect more severe stages of the disease. In these severe stages, beta-amyloid plaques and neurofibrillary tangles, two neurological indicators of AD, tend to aggregate more acutely than in previous stages. To help develop treatments, mouse models are utilized, typically. In order to test what type of model mouse could potentially be used to explore further treatment, the 5XFAD model mouse, one that produces an abundance of beta-amyloid plaques in its brain, was chosen. This paper intends to compare 5XFAD mice to the human brain in its severe stages of Alzheimer's through a qualitative comparative analysis to juxtapose the neurological factors of both brains. It also utilizes interviews in order to compare severe AD patients and 5XFAD mice phenotypically, and in terms of their cognitive and behavioral deficits. The author finds that the 5XFAD model is very limited as a medium to discover new treatments, and can only be used to a certain extent. Due to AD being defined by both the aggregation of beta-amyloid plaques and neurofibrillary tangles, but only beta-amyloid plaques being present in 5XFAD mice brains, treatments that target the beta-amyloids in the brain can be discovered due to the highly occurring similarities between the two, neurologically and behaviorally. However, a treatment that encompasses both contributing factors to Alzheimer's Disease will not be able to be found.

Introduction

The most common form of dementia, Alzheimer's Disease (AD), is increasing its prevalence across the country. AD has a widespread presence of 1 percent for those from 65 to 69 year-olds. This number rises with age; for 95-year-olds and older, AD has a presence of 40 to 50 percent (Nussbaum & Ellis, 2003). "Alzheimer's disease (AD)...typically manifests through a progressive loss of episodic memory and cognitive function, subsequently causing language and visuospatial skills deficiencies, which are often accompanied by behavioral disorders such as apathy, aggressiveness, and depression" (Ferreira Silva et al., 2019).

While being defined with loss of cognitive and behavioral function, AD is also characterized by the presence of increased beta-amyloid (amyloid β) plaques and neurofibrillary tangles that contain hyper-phosphorylated tau (Ferreira Silva et al., 2019). In recent years, treatment has been used to focus on the neurological aspects of AD (e.g., beta-amyloid plaques and tau phosphorylation); however, as Alzheimer's disease progresses, treatment goes to focus on treating symptoms, rather than slowing the deposition of these plaques and tangles (National Institute on Aging, 2021). While research towards late-stage AD treatment does not go unnoticed, there is very little movement into determining how to slow neurological changes, rather than physical changes.

Research towards treatment of AD primarily focuses on the experimental use of model mice in order to mimic the effects of this neurodegenerative disease. Model mice that are used for AD are a type of transgenic mouse that have genes that code for the over-phosphorylation of tau or over-deposition of beta-amyloid plaques (See Figure 1)

(Salazar et al., 2019). While there are a variety of transgenic mice used in order to generate different types of experimental treatment, 5XFAD model mice are common. 5XFAD mice are a type of transgenic mice that have a large overexpression of beta-amyloid plaques, mimicking the phenotype of an AD patient after 4-5 months of the mouse's lifespan (Amram & Frenkel, 2017). Characterized by this substantial number of plaques deposited into their brain and the cognitive or behavioral deficits that come along with that, 5XFAD mice are generally used to push forward innovative treatments.

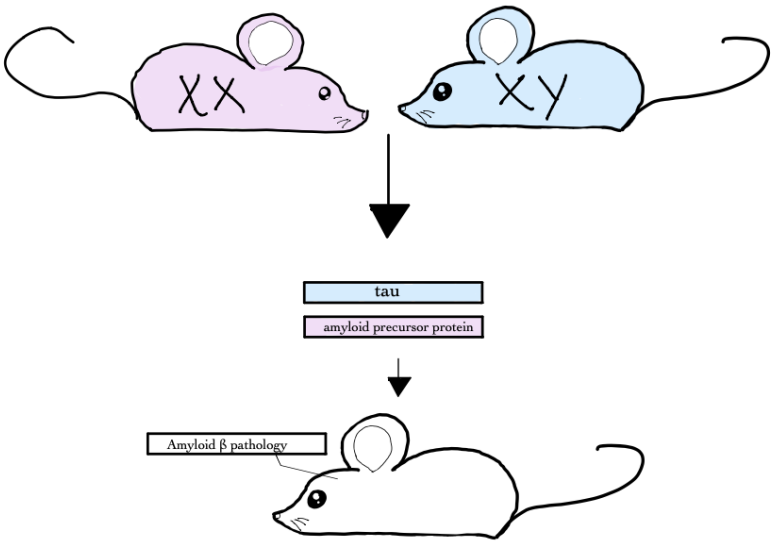

Figure 1. Illustration of the process of creating a transgenic mouse that contains the genes encoding for beta-amyloid overproduction and tau over-phosphorylation.

Gap in the Research

The pre-existing research connects 5XFAD model mice as a medium for progressing experimental treatment for Alzheimer's Disease (Gozes, 2016; Oblak et al., 2021). However, the pre-existing research does not address whether 5XFAD as a model mouse can be used for treatment of late-stage Alzheimer's, specifically. The purpose of this study is to investigate the extent of 5XFAD mice in determining this treatment. As there is very limited research on treatment for severe stages of Alzheimer's (National Institute on Aging, 2021), it is crucial to determine the link between 5XFAD mice and late-stage AD.

Literature Review

Neurological factors that contribute to Alzheimer's Disease

The pathology of AD can be attributed to many factors and does not have one specific cause. Different factors, such as disturbance of the cholinergic system of the body, hypoperfusion, lack of oxygen in the brain, calcium ion signalization problems, neuroinflammation, mitochondrial dyshomeostasis, protein misfolding, and decreased beta-amyloid clearance can be attributed to the onset of Alzheimer's in the body. Behind all of these factors, however, is one common component: increased beta-amyloid deposition within the brain (see Figure 2) (Penke et al., 2017). Many studies tend to corroborate that beta-amyloid deposition can be the most prominent cause behind the appearance of AD, which

is why most experimental treatments are directed toward inhibiting the proteins that bring about beta-amyloid plaques. Dr. Roland Jakob-Roetne and Dr. Helmut Jacobsen, two scientific experts at Hoffmann-La RocheKiel University, write that while the current methods for finding treatment for Alzheimer's Disease include a myriad of techniques, most of them are directed towards searching for inhibitors of the proteolytic enzyme β- and γ-secretase. These inhibitors would inhibit the separation of amyloid β precursor proteins into the amyloid β peptides (Jakob-Roetne & Jacobsen, 2009). These peptides eventually cause the deposition of the beta-amyloid plaques that lead to the onset of AD, so by inhibiting them, the peptides will impede the development of plaques in the brain.

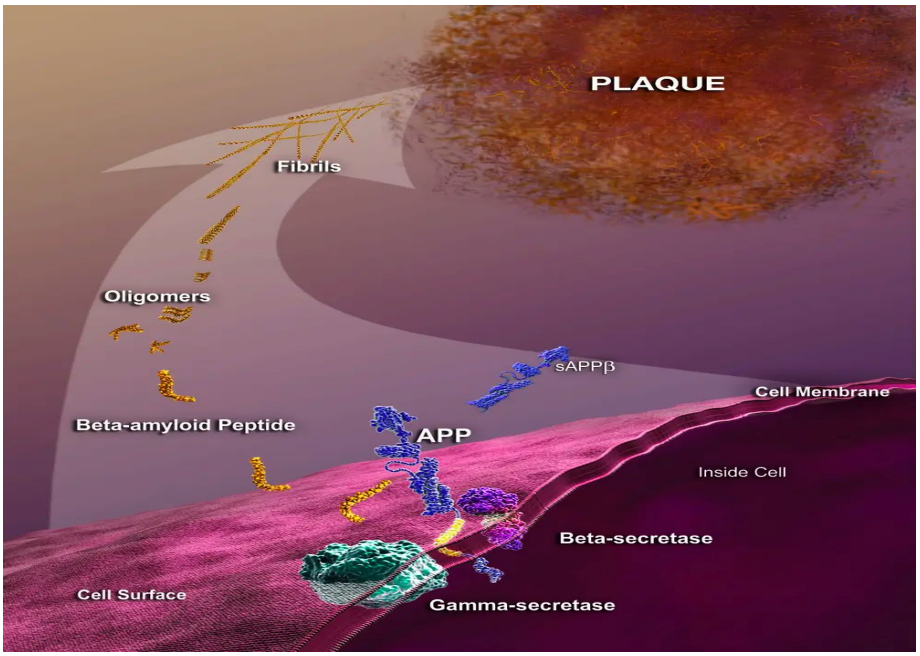

Figure 2. Illustration of the process of beta-amyloid plaques forming, starting from the expression of the APP protein, to the peptides accumulating to make the plaque, from (National Institute on Aging, n.d.).

While many of today's treatments toward Alzheimer's are directed using the amyloid hypothesis, others can disagree that beta-amyloids are the root cause of AD. According to Hashiro Sugimoto, who works in the Department of Neuroscience for Drug Discovery at Kyoto University, the amyloid hypothesis works to prove that the abundance of beta-amyloids in the brain is what causes the neurotoxicity that characterized AD (Sugimoto, 2010). In alignment with this, Thomasy (2021) finds that the beta-amyloid theory drives research as it is a hallmark of the disease. However, using the amyloid hypothesis can divert attention from a more holistic view of the disease, in that there are other major hallmarks of the disease, such as the aggregation of the protein tau (Thomasy, 2021).

Two researchers from the *British Medical Journal* corroborate Thomasy's findings, describing that while neuritic plaques are a major feature of Alzheimer's Disease, neurofibrillary tangles, caused by the accumulation of the tau protein, are also one main cause. The presence of both of these factors is what creates the symptoms that characterize Alzheimer's Disease (Burns & Iliffe, 2009). Einar M. Sigurdsson, a researcher at NYU Langone Health, (as cited in Thomasy, 2021) continues this: "Targeting tau is probably a more feasible approach in the later stages of the disease." Strongly connecting the works of Thomasy and Burns and Iliffe, Metaxas & Kempf (2016) write how neurofibrillary tangles (NFTs) are "fundamental" to the pathology of AD. Although researchers are unsure as to what exactly in the NFTs cause the neurotoxicity in the brain, the hyperphosphorylation of the protein tau, which is a major component of the NFTs, is connected to the memory and synaptic defects associated with AD.

Current use of 5XFAD model mice in determining treatment

In efforts to develop treatments for Alzheimer's, many types of mouse models are used. Each one is carefully designed toward a feature of the disease, based on what the experimental treatment is trying to target (Jankowsky & Zheng, 2017). To target beta-amyloid plaque deposition in the brain, a transgenic mouse model, the 5XFAD model, is popularly used. This mouse model shows the characteristics of the disease, such as "amyloid deposition, gliosis, and progressive neuronal loss accompanied by cognitive and motor deficiencies" (Oblak et al., 2021). However, neurofibrillary tangles are not present in this mouse model.

Continuing with this, researchers have utilized this mouse model for different causes. A popular use of 5XFAD mice is the targeting of beta-amyloid plaques and their agglomeration. A group of five researchers published in the International Journal of Peptide Research & Therapeutics, an international, peer-reviewed journal, experimented to determine the use of a skate skin hydrolysate in suppressing beta-amyloid formation (Lee et al., 2021). Similarly, 5XFAD mice were used in another experiment by a group of researchers to show how amyloid plaques can disassociate in the brain due to a chemical drug called Quinacrine (Park et al., 2021).

While some researchers focus on the suppression of amyloid plaques for their experimental use of 5XFAD mice, other researchers focus on the behavioral and cognitive deficits that 5XFAD mice develop. In *Genes, Brain and Behavior,* a peer-reviewed, scientific journal, a study was published describing the effect of the amyloid-plaque deposition on a specific behavior of mice: whisker movements. Allowing them to go through three tests, which were object exploration, sequential object exploration, and tunnel running, they found that while there were sex differences, there was still a significant alteration of this behavior in the 5XFAD mice (Grant et al, 2018). Likewise, others also experiment with the suppression of the cognitive deficits that appear in this model mouse. Using different tests, such as an open field, rotarod, Morris water maze, and Y-maze tests, researchers found that the effects of a cyclopentanone derivative helped enhance Alzheimer's memory and mitigate neuroinflammation (Ullah et al., 2020).

Although many investigate the effects of different treatments on mitigating neurological or behavioral factors of Alzheimer's Disease using this mouse model, some focus specifically on treatments regarding different stages of the disease, popularly the early stages of AD. Published in *PLOS One*, a scientific, peer-reviewed journal, Jović et al. (2019) found that while investigating the effects of fish oil supplementation through 5XFAD mice, its consumption may help improve the AD pathology, but only in the presymptomatic stage. In contrast, others explore other stages of the disease. Four researchers published in *Genes, Brain and Behavior,* found that motor impairments had a positive correlation with age, exemplifying how as plaques in the mice build up, the mice show continued impairment and dysfunction (O'Leary et al., 2018). However, while this study does show moderate to late-stage behavioral impediments, it did not include treatments that may occur to ameliorate these impediments.

Current Treatment for Late-Stage Alzheimer's Disease

Currently, the treatment towards severe AD is very limited, as most efforts towards treatment are attempting to target the presymptomatic stages of this disease. However, researchers have made strides in creating new treatments for those in the later stages of Alzheimer's.

Donepezil is one of the two drugs approved by the Food and Drug Administration (FDA) to treat late-stage AD patients. While it does not target amyloid plaques, Donepezil works to inhibit acetylcholinesterase in the brain (Bryson & Benfield, 1997). Acetylcholinesterase acts toward the death of cholinergic neurons and leads to deficits in nerve cell communication (Rees & Brimijoin, 2003). By inhibiting them, treatments work to restore synaptic levels of the neurotransmitters, acetylcholine, and stopping acetylcholine turnover. Published in the *CNS Neuroscience & Therapeutics*, an open-access, medical journal, four researchers found that Donepezil works to alleviate cognitive and global functioning; however, after being treated with high dosages, those patients discontinued their use of the medication due to treatment-related adverse events (Adlimoghaddam et al., 2018).

While some turn to the singular use of medications such as Donepezil to treat severe Alzheimer's, other holistic views are approached. 6 neuroscientists published in *Alzheimer's Research & Therapy* investigate the use of memantine monotherapy and a combination of memantine and Donepezil. These researchers found that although the use of memantine monotherapy helped alleviate the symptoms, it was the combination with Donepezil that had the additive effect for moderate to severe AD (Atri et al., 2015).

Even though cholinesterase inhibitors are the standard for treating Alzheimer's, new non-standard treatments are also starting to emerge. Srdjana Telarovic and Alisa Junakovic, neuroscience researchers at the University of Zagreb, found that unconventional methods such as art therapy, music and dance therapy, clay and manipulation therapy, and tai chi therapy do have promising results to help the motor and non-motor symptoms of AD (Junakovic & Telarovic, 2021). Agreeing with this, Deygout & Auburtin (2020) found that a non-pharmacological approach, art therapy, aids severe-stage Alzheimer's patients, as it acts as a sensory stimulation intervention technique, increasing pleasurable feelings.

Methodology

Study Design and Rationale

This study was designed in order to answer the question: "To what extent can 5XFAD model mice be used to determine experimental treatment for late-stage Alzheimer's Disease?" The objective of this study design was to investigate the usage of these transgenic model mice and see whether experimental treatment for late-stage AD, in which there has been a low prevalence, can be generated from this usage. I hypothesized that due to the similar nature of the 5XFAD mouse brains and human AD patients' brains, 5XFAD mice can continue to be used as a tool in discovering new treatments for late-stage Alzheimer's.

To confirm or reject my hypothesis, a two-part qualitative study was utilized. This allowed for both neurological and behavioral reasoning behind my conclusion. This is important because just focusing on one aspect, such as behavioral, would limit the understanding of new treatment to only treating behavioral aspects of late-stage AD. The two methods that were utilized to develop this understanding were: semi-structured qualitative interviews and a qualitative comparative secondary data analysis. Through the literature review, it is seen that many of the methods used to analyze Alzheimer's Disease and 5XFAD model mice are through quantitative experiments, such as preparing histological sections of both human and model mouse brains and analyzing the number of beta-amyloids in their brain. They also utilize observational experiments where they observe the mouse behaviors and compare them to human behaviors. Due to certain limitations, such as the unavailability of a lab, an experiment was not able to be done. However, qualitative interviews and a qualitative comparative secondary data analysis allow for a similar level of conclusivity, as the interviews allow for an insight into animal behavior from those who have experienced it firsthand, and the qualitative comparative secondary data analysis allows for the comparison of imagery of human beta-amyloid plaques and mouse plaques, which hasn't been previously done in studies.

Semi-Structured Qualitative Interviews

To complete the first part of my study, I conducted semi-structured interviews. "The method allows the researcher to collect open-ended data, to explore participant thoughts, feelings and beliefs about a particular topic and to delve deeply into personal and sometimes sensitive issues" (DeJonckheere & Vaughn, 2019). It was imperative that the interviews were semi-structured in order to have a full understanding of the animals' behaviors, in case the questions that were provided did not cover everything about the behavior.

The interview questions in the study were based on pre-existing research, and what behavioral factors are involved in characterizing Alzheimer's Disease in humans. The questions were not only based on late-stage Alzheimer's Disease behaviors but on moderate stages, as well. These were reviewed and approved by the Institutional Review Board (IRB). The interview questions used in the study are located in Appendix A.

Subjects were individuals that had worked with and done observational experiments with 5XFAD mice in past papers. The pool of these individuals was composed of head researchers, professors, graduate students, and lab assistants. This pool was chosen because they have firsthand experience with 5XFAD mice and they have an understanding of the animals' behavioral progression. Potential subjects were collected by compiling a list of the information of researchers who have worked with the mice, through papers they have published (n=25). After compiling a list of all the subjects, each was emailed, with the consent form attached (see Appendix B).

As defined by the Medical Research Council, research fraud is the falsification or plagiarism of research results (MRC, 1997, as cited in Gupta, 2013). In order to eliminate issues of research fraud or ethical issues, the IRB approved the consent form, which allowed the subject to choose whether their name will be included, as it is their research, and if they are able to be recorded. In the email, they were given the option of doing the interview in person, over the phone, or a video conference. If emailed back, a time and date were scheduled to conduct the interview. If the subject chose to be interviewed over a video conference, the invitation was sent out in advance of the time and date scheduled, in order to eliminate scheduling conflicts. When conducting the interviews, the standardized interview questions were asked; however, if further questions about the neurobiological factors or behavioral factors in 5XFAD mice arose, they were asked. After the interview finished, the recording was transcribed (see Appendix C) and analyzed based on general themes.

Qualitative comparative secondary data analysis

Walk (1998), for the Writing Center at Harvard University, writes about how a comparative analysis weighs A and B equally. A and B may have differences, but they also have commonalities that can be hidden. In the comparative analysis conducted in this study, the two subjects being compared are the imagery of 5XFAD model mice brains throughout their lifetime, and the imagery of human AD patients, through their later stages of AD.

Sources were located through three scholarly databases: EBSCO, Jstor, and Google Scholar. Two separate search processes were conducted in order for data collection to have both 5XFAD imagery and AD human imagery.

The first search process was conducted towards finding 5XFAD brain imagery. After limiting keywords to 5XFAD mice, Alzheimer's, histological sections, and beta-amyloid plaques (using the Boolean Operator "AND" for every term), every other source was selected to scrutinize. Sources were scrutinized based on if they had imagery and what type of imagery. If the imagery did not show beta-amyloid plaques or full-brain imagery, that source was not chosen as a part of the study. Sources that were chosen as a part of the study were kept securely in a document until data analysis.

The second search process was similar to the first; however, this was dedicated toward finding severe-stage Alzheimer's patients' brain imageries. After limiting keywords to severe Alzheimer's, histological sections, beta-amyloid plaques, and human brain (using the Boolean Operator "AND" for every term), every other source was selected to scrutinize. Like the first search process, if the imagery did not show beta-amyloid plaques or full-brain imagery, that source was not chosen as a part of the study, and the sources chosen were kept securely in a document until data analysis.

Data analysis was conducted qualitatively; general features that seemed to arise from the imagery of humans and the imagery of mice, individually, were recorded. After identifying the features of the imagery, they were cross-analyzed to discover any major comparisons that arose from the differing subjects. The overlapping features (major comparisons) acted as groups, and sources that corroborated them were quantified.

Results

Semi-structured Interviews

Although only one interview was able to be conducted, much qualitative data about the behavior of the model mice was collected. From the interview, three general themes arose: cognitive or behavioral deficits, motor deficits, and the limitations of neurobiological deficits.

The first theme, which was cognitive or behavioral deficits, showed some similarities to human Alzheimer's Disease. When asked about the behavioral phenotype of the mice, Researcher A stated that there were tests done to catch sight of the deficits that may occur from the over-deposition of plaques in the 5XFAD mice brain. By first doing the task of the open field test, where mice are put in a large box, the researcher observed, "But what we've experienced is that in the 18-month time point, not only are there not really much differences in the way that healthy or 5XFAD mice kind of spend time in the center." While the mice did not show much of a behavioral deficit, speed and distance differences were shown in other tests. In a test called the "Elevator Plus Maze," the researcher states, "...the 5XFAD mice actually, for whatever reason, have more time spent in the open arms compared to the healthy control, which could kind of suggest some sort of issue in the way that it's kind of processing its environment a little bit."

The second theme that was conceived from the interview is the similarities in motor deficits. While they do have some cognitive deficits, 5XFAD mice also showed major motor impairments. In the open field test mentioned before, the researcher found that:

> As I mentioned in the open field test, they traveled fewer distances compared to the wild-type healthy controls, and their speed was also reduced, which is interesting because you would think that because they traveled smaller distances and they had a reduced speed, you would expect that maybe their body mass was a little too heavy when, in fact, the weight actually decreased compared to healthy control. And so most likely what I'm inferring, at least, is that perhaps the 5XFAD mice were having perhaps a slower reaction or slower processing of its environment.

This evidence generated from the researcher's observations exhibits that there was both a mix of cognitive and motor impairments in 5XFAD mice, due to a lack of awareness of surroundings, or a slower reaction time.

The third theme that was brought about from this interview is the limitations of the neurobiological factors. The researcher explains that in reality, it is the combination of another factor, tau phosphorylation, that contributes to the defined phenotype of Alzheimer's. As Researcher A states, "And that's kind of like the flip side of Alzheimer's is that you need both tau and Alzheimer's among the plaques to really cause the brain atrophy and dementia cognitive deficits there." While the beta-amyloid plaques in the brains of the 5XFAD mice are connected with impairments in cognition, the researcher explains the limitations behind the comparison of the deficits, which is that the two subjects cannot be compared fully, as the human has the factor of tau contributing to behavioral deficits, while the mouse model does not.

Qualitative comparative secondary data analysis

After applying a comparative analysis to the different imaging I collected, two major comparisons emerged: the area of the deposition of beta-amyloid plaques and the aggregation of plaques spotted (see Table 1).

The first comparison was the location of the accumulation of beta-amyloid plaques. In both human and mouse brains, beta-amyloid plaques can be deposited in different parts of the brain, but where they are mainly found is in 5XFAD and in human brains is the hippocampus. While different imagery showed beta-amyloid accumulation is higher throughout most of the brain, not just clustered in one spot, the numbers of plaques that accumulate in the hippocampus region show lesser, but similar numbers, as compared to the whole brain. It is important to note, however, that some imagery deviated from this notion, as it was limited to the brain as a whole, not specific regions.

The second comparison that emerged was the quantification of the plaques in the brain. While compared qualitatively, due to the inaccessibility of an experiment, the numbers for the plaques in the brain for 5XFAD histological sections were very similar to that of human AD brain images, especially those in moderate to severe stages. In earlier stages of life, the mouse model showed presymptomatic levels of plaques in their brains, but imagery showing the progression of the mice through their lifetime exhibits similar numbers of plaques in the brain compared to moderate to severe-stage Alzheimer's patients.

Table 1: Frequency of secondary images that exhibit the two major comparisons found in the comparative analysis conducted.

Comparison	Comparison Understanding	Number of sources that corroborate this comparison
Areas where most plaques are primarily found	In both 5XFAD mice brains and human AD patient brains, an area where many of the plaques tend to aggregate is the *hippocampus*.	4
Aggregation of plaques in the brain	As the transgenic mice grow older, they tend to accumulate more amyloid plaques, similar to the number of late-stage human patients.	5

Discussion

As stated previously, when comparing data from the brains of severe-AD patients and the brains of 5XFAD mice, two major comparisons emerged.

As depicted in Table 1, four sources confirm that in both 5XFAD and late-stage AD humans, amyloid plaques tend to grow in numbers in the hippocampal region. This finding emphasizes that the brains (one of the mice and one of the human) are very similar in nature, in terms of where the plaques can be deposited. This confirms that while beta-amyloid plaques are one of many factors to be focused on, to focus on them for new treatments would bring results due to the likeness of the two brains.

Not only do 5XFAD mice and AD patients have an agreement on the location of where they are deposited, there can also be a comparison in terms of aggregation. As seen in Table 1, one of the two main comparisons that emerged when completing the analysis is the similarities between the number of plaques deposited in 5XFAD mice brains and an Alzheimer's brain. Based on the results, while in early stages, these transgenic mice do not mimic the large number of plaques deposited in the human brain, as they grow older, similarities between severe Alzheimer's and 5XFAD mice start to appear. Due to the nature of the 5XFAD brain, these mice tend to over-deposit beta-amyloid plaques, meaning that they start to grow in large numbers as time goes on. Likewise, Kooresh et al. (2002) found in their study that as Alzheimer's progresses in the human brain and becomes more severe, the plaques that are deposited grow in number and are slower to clear. This finding suggests that because the brain of the transgenic mouse and the brain of the severe AD patient correspond, in terms of the numbers of deposits, this makes 5XFAD mice a good model to test experimental treatments for humans on.

Another finding that appeared when conducting my study is the similarities between the 5XFAD model mice and AD patients, behaviorally. As said in the interview conducted, "There does seem to be some cognitive deficits, behavioral deficits, but more closer to the 18 months age…even if it does occur, it does not occur until the later, later

stages." This researcher then goes on to describe the different tests that were done to determine such behavioral deficits, such as a contextual fear conditioning task and a road task, which showed that the animal's behavior and cognition deteriorated with amyloid plaques, as with human severe-AD patients, too. This finding shows while similarities arise neurologically, they also can arise behaviorally, as AD patients in their severe stages of Alzheimer's tend to also have major cognitive deficits, unable to complete daily tasks or activities (Galasko, 2005). The interview, however, brought a limiting factor to the new understanding. The last major theme that arose from the interview was the presence of other neurological factors that affect the behaviors of human patients. Tau phosphorylation, as Researcher A agrees, also plays a part in distinguishing AD and its symptoms, meaning that the symptoms shown in 5XFAD mice are not fully equal to the symptoms in human AD patients, as 5XFAD mice do not express tau phosphorylation. These findings agree with Thomasy (2021), who found that driving treatments for Alzheimer's behind the amyloid hypothesis is not realistic, due to the presence of other neurological factors, like neurofibrillary tangles.

Conclusion

Alzheimer's Disease is a neurodegenerative disease that is in desperate need of extended research on novel treatments. Because it is such a vast disease that is hard to pinpoint a specific cause to, research on the full extent of it is very limited, especially for severe stages of Alzheimer's (Voisin & Vellas, 2009). This study focuses on the use of a specific type of transgenic model mice, 5XFAD mice, to determine new treatments for these severe stages of Alzheimer's.

The hypothesis that 5XFAD mice can be used as a tool in discovering new treatments for late-stage Alzheimer's is somewhat supported by the data collected. Returning to the pre-existing research, this model mouse is known to demonstrate an abundance of beta-amyloid plaques in the brain (Amram & Frenkel, 2017; Oblak et. al, 2021; Sugimoto, 2010). After analyzing the data collected, the study was able to conclude that 5XFAD mice can be used to a certain extent for developing an experimental treatment, in terms of targeting beta-amyloids, due to the similarities of the plaques in location, number, and the similarities in cognitive, behavioral, or motor deficits between the mice and the severe-stage AD patients. However, due to the emergence of tau as another neurodegenerative factor influencing behavior and neurotoxicity, 5XFAD mice cannot be used to a full extent to determine complete late-stage Alzheimer's treatment and are limited to targeting plaques.

Implications

This study was intended to help Alzheimer's patients in severe stages of their disease by situating the use of 5XFAD model mice into severe stages of Alzheimer's. The findings of this study can generate new initiatives towards new treatments for late-stage Alzheimer's, as those that were not informed of the comparison between severe-stage patients' plaque buildup, and the plaque buildup in later-age 5XFAD model mice can now use 5XFAD as a medium to develop treatments. In addition, the National Institute on Aging (2021), writes that the research on late-stage Alzheimer's is very limited, and is more focused on mitigating the symptoms of the disease, rather than the root cause. For this reason, this study can spur new efforts using 5XFAD mice, not only for severe-stage Alzheimer's Disease but also for other stages, especially using earlier ages of the mice.

Limitations

This study, however, is only conclusive to a certain extent. One limitation to this is the small sample that was generated from the interviews. While emails requesting interviews were sent out to a large pool of researchers who have worked with model mice, including professors, lab assistants, or graduate students, only one response was received. While this response is corroborated by other sources, the sample size is not enough to generate quantitative results, limiting

the study to qualitative results. This also makes the conclusion less generalizable as the behaviors of 5XFAD mice may vary in different environments and in different tests, limiting the study's behavioral results.

Secondly, a large limitation of this study would be the focus on beta-amyloids. Due to 5XFAD mice only coding for overexpression of beta-amyloids, this leaves out the significant aspect of tau hyperphosphorylation, which can also play a big role in defining Alzheimer's disease. As said in the interview conducted, while beta-amyloids do produce similarities between model mice and human brains, behaviorally and neurologically, the hyperphosphorylation of tau with the over-depositing of the amyloid plaques are what truly characterize AD, and the deficits that come along withthe both of them. Similarly, Thomasy (2021) used this reasoning of tau phosphorylation as a way to limit the amyloid hypothesis and experiments that are driven by this hypothesis.

Furthermore, another limitation lies in my methodology. While multiple databases were utilized in order to collect the sources, when collecting data for the secondary data analysis, I chose to do random sampling, picking every other source and scrutinizing it for information. However, while doing random sampling, this may leave out sources that can have imagery relevant to my data.

Fulfillment of the Gap in the Research

The findings from this study address the gap in the research. First, the gap in the research was the lack of a link between 5XFAD model mice and experimental treatment toward severe stages of Alzheimer's Disease. In pre-existing studies, there was not much progress toward the development of treatment for severe-stage patients. However, also prevalent in the pre-existent research is the lack of specification for 5XFAD mice, and if they can be used to target a specific stage. This study bridges the gap between the two by the comparison of both neurological and behavioral aspects of the 5XFAD mice and comparing those to specifically humans in late-stages of Alzheimer's. This fills the gap by both specifying that 5XFAD mice can be used to progress late-stage treatment and exhibit new mediums to push forward treatments for the underrepresented stage of AD.

Areas for Further Research

While the study did have its limitations, further research can be expanded to create more validity for the usage of 5XFAD model mice. While this study primarily focused on secondary data analysis and interviews, the research can be extended to include a quantitative aspect in order to encompass everything about the usage of 5XFAD mice. Conducting this same research with the image capturing of histological sections of 5XFAD mice at different stages of their life would help quantify the beta-amyloid plaques, and allow us to compare them to the number of plaques in human brains.

The limitations in this study also act as catalysts for further research. As seen from the results and pre-existing research, amyloid plaques are not the only contributors to neurotoxicity and cognitive impairments (Burns & Iliffe, 2009). Continuing with the research for beta-amyloids, including model mice that also have hyper-phosphorylated tau would make it so that there is a clearer understanding of the treatment. Since Alzheimer's is defined by both the tau phosphorylation and the beta-amyloid plaques, using mice that have an abundance of hyperphosphorylated tau may bring about better mediums for new treatment.

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