The effect of caffeine on the reduction of amyloid-beta aggregation in late-onset Alzheimer's disease

AP Research

May 2019

Word Count: 4379

Abstract

In this study, the effect of caffeine on the concentrations of amyloid-beta proteins present in patients with late-onset Alzheimer's disease was investigated. The two types of these proteins, amyloid-beta 1-40 and 1-42, are known to aggregate and cause plaques among neurons throughout the brain, disrupting neuron function and therefore inhibiting cognitive abilities that translate to the rest of the body. Data on transgenic mice experimentation was obtained through systematic literature review, and results of a statistical analysis expressed a negative correlation between the concentration of caffeine and concentration of amyloid-beta, suggesting that caffeine may play a role in reducing the levels of amyloid-beta proteins in the brains of patients with late-onset Alzheimer's disease.

Introduction

Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disease currently affecting close to six million Americans. It is the sixth leading cause of death with a 145% increase in recorded cases and no apparent cure (Alzheimer's Association, 2019). The disease itself is characterized by neuron death, which prevents nerve communication and signaling in the brain, reducing brain activity (Bright Focus Foundation, 2017). The disruption in cognitive function stemming from cell death and hampered communication is caused by the accumulation of harmful proteins that have the tendency to cluster together, forming extracellular plaques and intracellular neurofibrillary tangles (Murphy & LeVine, 2010). Being that the aggregation of these toxic proteins occurs both between and within neurons, it very easily prevents neurons from sending

signals to each other and through the rest of the central nervous system (National Institutes of Health, 2016). As a result, the development of the disease affects both the brain and the body.

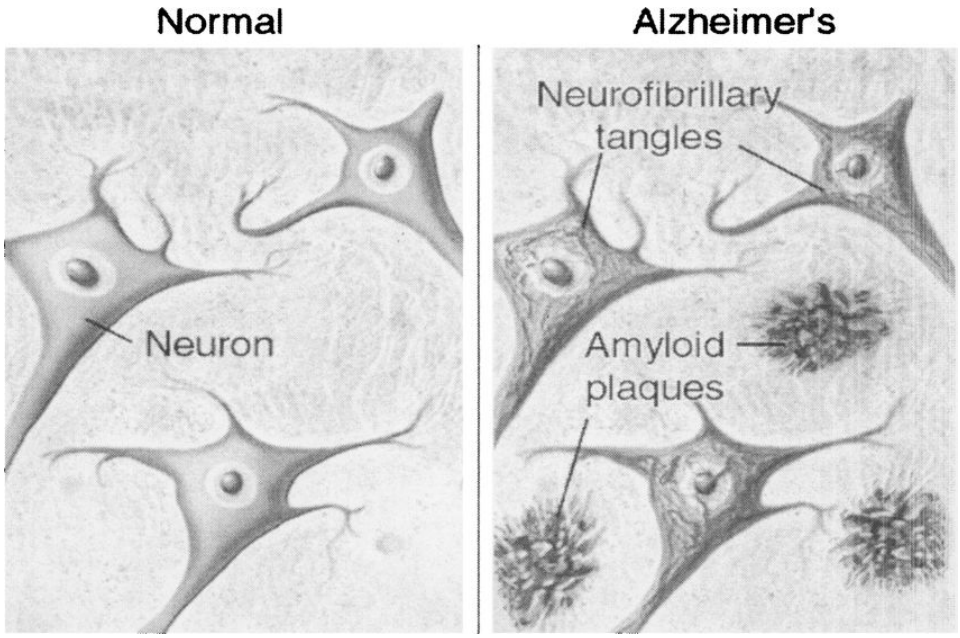

Fig. 1 Neurons in a normal brain and an Alzheimer's-affected brain shown side by side. Neurons in the Alzheimer's brain are interlaced with amyloid-beta plaques, and the insides of the cells have tangles (American Health Assistance Foundation, 2004).

Types of Alzheimer's

The most prominent type of Alzheimer's disease is late-onset Alzheimer's, comprising 97% of all cases in the United States. Symptoms typically become present in people aged sixty to sixty-five (Alzheimer's Association, 2019). Because aging is associated with the natural deterioration of the brain, it is typical for the risk of Alzheimer's to increase with age; at over sixty-five years old, a person's risk of developing the disease doubles every five years (Isik, 2010). The remaining 3% of Alzheimer's cases fall under early-onset, where symptoms develop

in patients under the age of sixty-five. Early-onset Alzheimer's is known to have genetic markers increasing the susceptibility of the brain to develop causes of the disease (Koedam et al., 2010).

Symptoms and Progression

The main symptoms of Alzheimer's negatively affect brain function and communication throughout the central nervous system. The resulting cognitive impairment causes the loss of memory, critical thinking, judgment, reasoning, and physical ability, as well as the onset of psychological and behavioral problems (National Institutes of Health, 2016). The severity of these general symptoms depends on the progression of the disease; the higher its progression, the worse the symptoms become.

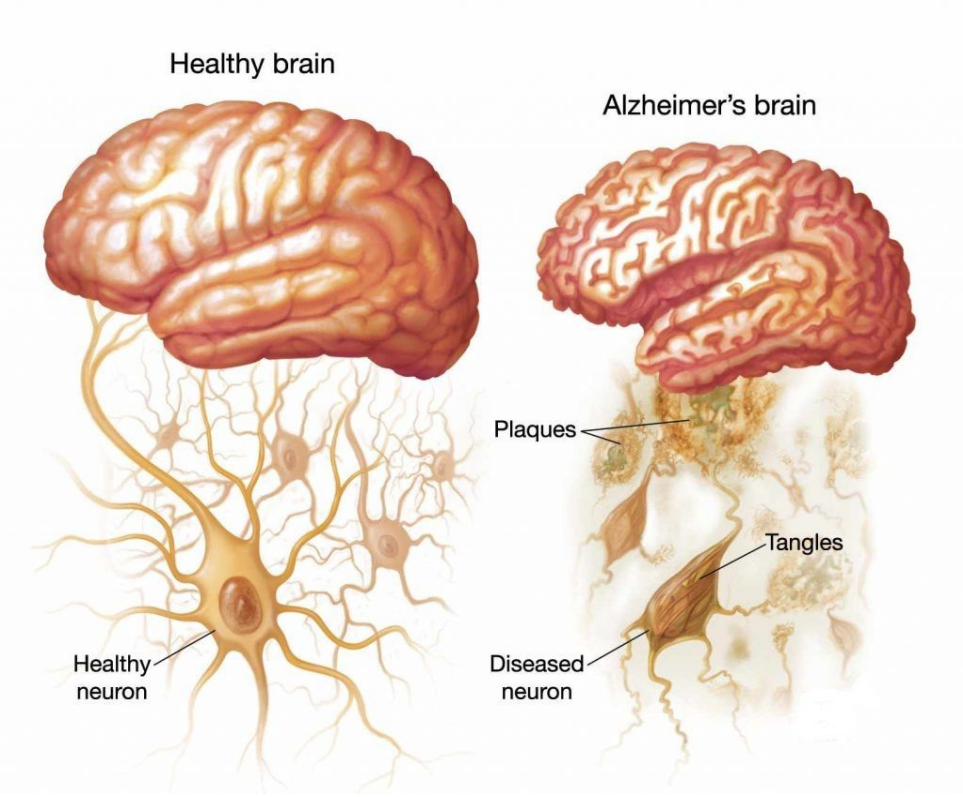

Fig. 2 The effects of tangles and plaques from Fig. 1 on neurons and the brain. Affected neurons become diseased, axon connection is blocked, and brain mass decreases as a result of apparent neuron nonfunction. Typical symptoms of Alzheimer's disease are then induced. (Allied Academies, 2019).

Alzheimer's disease progresses in four main stages. The first, preclinical, is characterized by changes occurring in the brain before symptoms show and can last for varying amounts of time, typically many years (Johns Hopkins Medicine, 2019). During this time period, noticeable symptoms of Alzheimer's are not present, but developments of the disease, including harmful protein buildup resulting in the tangling and blocking of affected neurons, take place. The length of the preclinical stage may be affected by social and environmental stressors ranging from mental and physical activity to changes in the molecular level that aid the brain in compensating for damage caused by plaque buildup (Very Well Health, 2018). Once the brain is no longer able to tolerate the onset present in the preclinical stage, the disease progresses to the early stage. During the early stage, patients experience mild symptoms of Alzheimer's, primarily short-term memory trouble, difficulties in organizational skills, misplacing or losing objects, mild mood swings, and growing anxiety, but are self-aware and able to recognize such shortcomings (Alzheimer's Association, 2019). Following further progression and increased plaque buildup, neuron function becomes increasingly compromised and moderate onset of the disease is initiated (Johns Hopkins Medicine, 2019). Typically the longest period of Alzheimer's development, symptoms are more pronounced than previous stages and patients have greater difficulty performing day-to-day tasks involving bodily coordination. Memory loss is more apparent, and personality and psychological differences become noticeable. Many patients in the moderate stage have problems recognizing familiars, experience paranoia, behave impulsively, are agitated, and have extreme mood swings (Alzheimer's Association, 2019). The last stage of Alzheimer's disease is the late to severe stage, where present symptoms worsen and physical changes occur. As plaques and diseased neurons become common throughout the brain,

cognitive function degenerates and patients lose bodily control and upkeep (National Institutes of Health, 2016). During this stage, patients lose both conscious and unconscious control over themselves as the brain is unable to regulate the body internally. At this point, patients are unable to communicate and experience loss in motor control, increased fatigue, loss of bowel and bladder control, little to no memory function, hallucinations, and seizures. Because unconscious body control shuts down, the immune system is weakened and as a result, most patients will die at the severe stage from the onset of viral or bacterial infections (Alzheimer's Association, 2019).

Social Effects

In addition to direct health detriments, Alzheimer's disease affects both patients and healthy citizens nationwide through caretaking and federal work that take time and monetary resources to fund. Over $350,000 per patient is put into providing individuals living with the disease with adequate care (National Institutes of Health, 2016). Due to its effects on the ability to control the body, patients living with moderate to severe Alzheimer's typically seek personal care to aid in aspects of daily life, including physical activity, eating, and bowel movements. Eighty-three percent is unrecorded and unpaid, but approximated at 18.5 billion hours and $234 billion annually (Alzheimer's Association, 2019). Additionally, Alzheimer's disease poses a cost to the government through federally-funded healthcare institutions. The establishment of hospitals, hospices, research institutes, and healthcare organizations Medicare and Medicaid totaled $277 billion in 2018, is expected to reach $290 billion in 2019, and is projected for $1.1 trillion in 2050 (Alzheimer's Association, 2019). The already detrimental effects of Alzheimer's

disease are only cemented by its far-reaching influence into the social and economic spheres of the United States.

Amyloid-Beta Proteins

The proteins that make up toxic extracellular plaques in Alzheimer's disease are amyloid-beta proteins. Their structure allows them to clump together to form aggregations of proteins that are large enough to disrupt neuron function (Hardy & Selkoe, 2002).

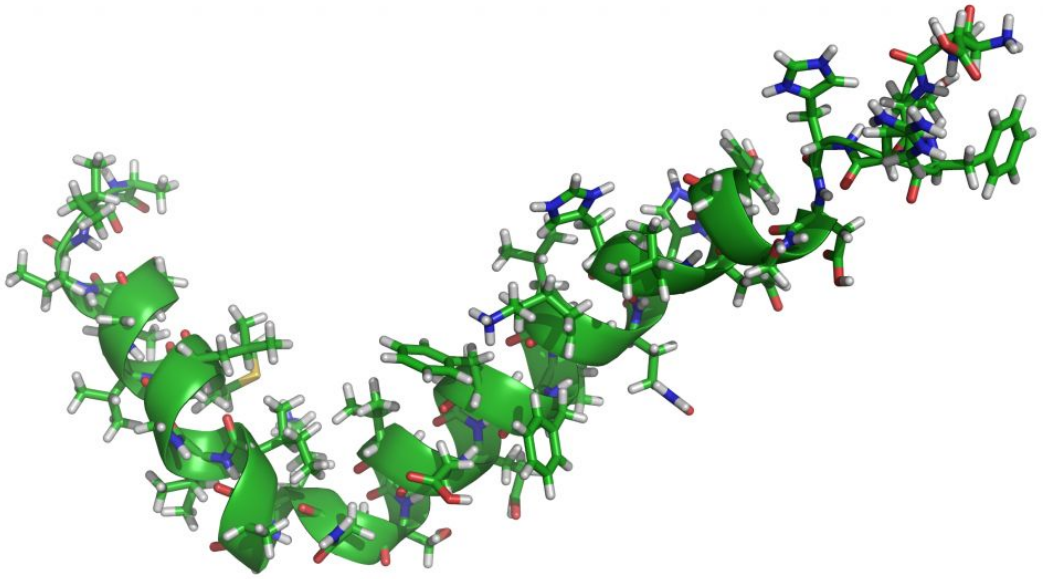

Fig. 3 Three-dimensional structure of amyloid-beta protein. Its shape and structure allows it to clump together to form increasingly complex protein plaques. (Meyer et al., 2017)

Amyloid-beta are formed from a larger parent polypeptide, amyloid precursor protein (APP), that is found in the central nervous system. Normally, it functions as a membrane protein regulating neuronal development, signaling, intracellular transport, and behavior (Chen et al.,

2017). Starting the formation of amyloid-beta plaques, the enzyme beta-secretase cuts amyloid precursor protein, cleaving it into soluble APP variants and terminal protein fragments. The enzyme gamma-secretase then cuts the terminal protein fragments into amyloid-beta peptides (Zheng & Koo, 2011). Since amino acids have one variant molecular group, an R-group, that actively interacts with R-groups of other amino acids, the interactions of amino acids in a protein determine its shape and function. Once singular amyloid-beta peptides are cut, they begin to change shape from such molecular interactions. The amino acid sequence of an amyloid-beta peptide is its primary structure. R-group interaction between amino acids gives it a two-dimensional secondary structure, where the protein strand is twisted into spring-like alpha helices, followed by strips of beta-sheets. Further interaction produces three-dimensional tertiary structure, this case being oligomers formed by the twisting of beta-sheets. Individual oligomers fold upon each other to reach quaternary structure, the arrangement of multiple folded proteins, forming fibrils. Large numbers of fibrils interact and aggregate together into complex clusters that form the amyloid plaques present in Alzheimer's disease (Zheng & Koo, 2011).

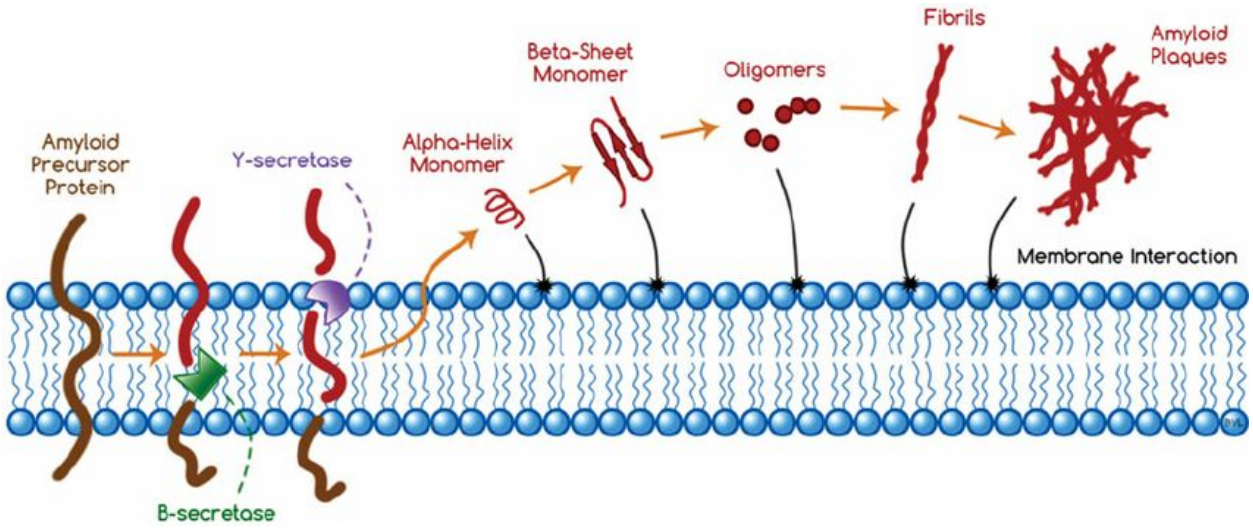

Fig. 4 The making of amyloid-beta plaques, starting from amyloid precursor protein, from which amyloid-beta is formed. Successive protein interactions and folding lead to the accumulated aggregation of amyloid-beta, and consequently the formation of plaques. (Drolle, et al., 2014)

From the cutting of amyloid precursor protein, five types of amyloid-beta can be formed, ranging from a numerical sequence of thirty-six to forty-three amino acids. In plaques, amyloid-beta 1-42 and amyloid-beta 1-40 are the most prevalent types of the protein, with their namesake reflecting sequences of forty-two and forty amino acids, respectively. Although their sequences differ by two amino acids, both amyloid-beta 1-42 and 1-40 have neurotoxic behavior and directly related to Alzheimer's disease progression (Qiu et al., 2015).

Caffeine and Molecular Antagonism

Caffeine is a stimulatory molecule commonly found in the beverages coffee and tea. Once ingested and present in the brain, caffeine acts as a molecular antagonist by actively blocking other molecules from binding to their own receptors. Part of caffeine's chemical

structure is similar in shape and atomic makeup to that of adenosine, a molecule found in the brain that aids in energy transfer and regulation, allowing caffeine to be accepted at otherwise adenosine-specific receptors (Ribeiro & Sebastião, 2010). Once caffeine binds to adenosine receptors, adenosine is blocked from binding and transmitting a signal to be communicated throughout the brain.

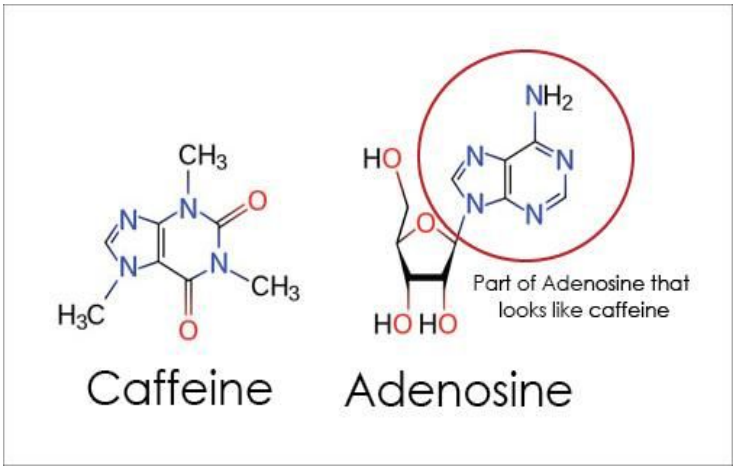

Fig. 5 Diagram showing the similarities between caffeine and adenosine molecules. Due to the nitrogen groups on both molecules, caffeine is able to bind to adenosine receptors and disrupt the circadian cycle, shifting bodily functions and brain behavior as well as incurring hyperactivity among neurons. (Dhinesh, 2017).

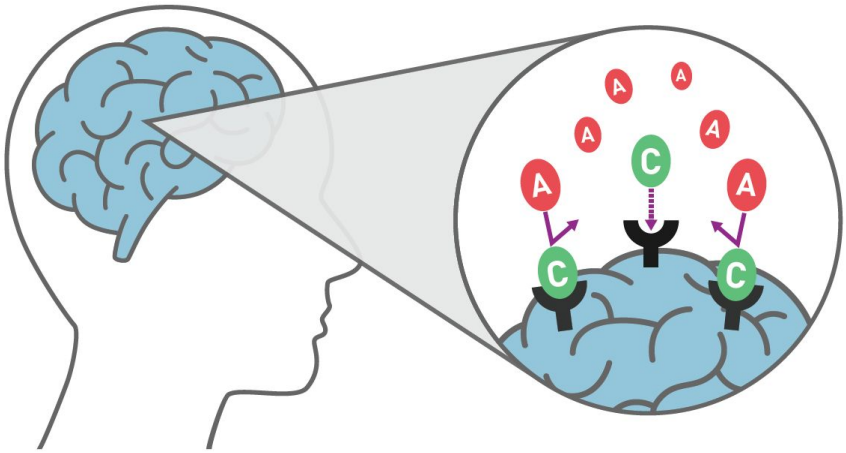

Fig. 6 Model of caffeine-adenosine antagonism. Caffeine is shown blocking adenosine molecules from binding with adenosine receptors as a result of the similarities between the two molecules in Fig. 5. (Institute for Scientific Information on Coffee, 2019)

Once caffeine binds to adenosine receptors, it is able to induce its own effects through neuron communication throughout the brain and to the rest of the central nervous system. Since caffeine is one of the most widely consumed pharmacologically active substances in the United States, it was previously assumed that the ingestion of caffeine had negative effects on the body, including cardiovascular detriments, calcium imbalance, increased risk of developing cancer, and general cell toxicity. Recent studies, however, have disputed such assumptions by finding caffeine to have neuroprotective effects, especially concerning the reduction of cognitive decline (Nawrot, 2010; Ritchie, 2007). Studies measuring the results of caffeine consumption and presence in the brain have shown there to be anti-inflammatory, anti-oxidative, and disease-preventative effects. Through molecular antagonism, it suppresses pro-inflammatory cell mediators to reduce detrimental autoimmune responses, prevents cell damage and protein

degradation from free radicals, and decreases the risk of developing chronic diseases by removing toxic buildup in the brain (Yenisetti et al., 2016). Clinical trials tracking caffeinated drink intake, specifically coffee and tea, showed overall increases in cognitive function through problem-solving and reasoning or decreases in cognitive decline in patients taking caffeinated drinks compared to control groups not taking them (Eskelinen & Kivipelto, 2010). These health benefits are being researched to determine if caffeine has a noticeable effect on reducing the magnitude of the currently incurable Alzheimer's and Parkinson's diseases, as they both stem from the buildup of toxic plaques in the brain that disrupt the communication and well-being of neurons, and both have no cure.

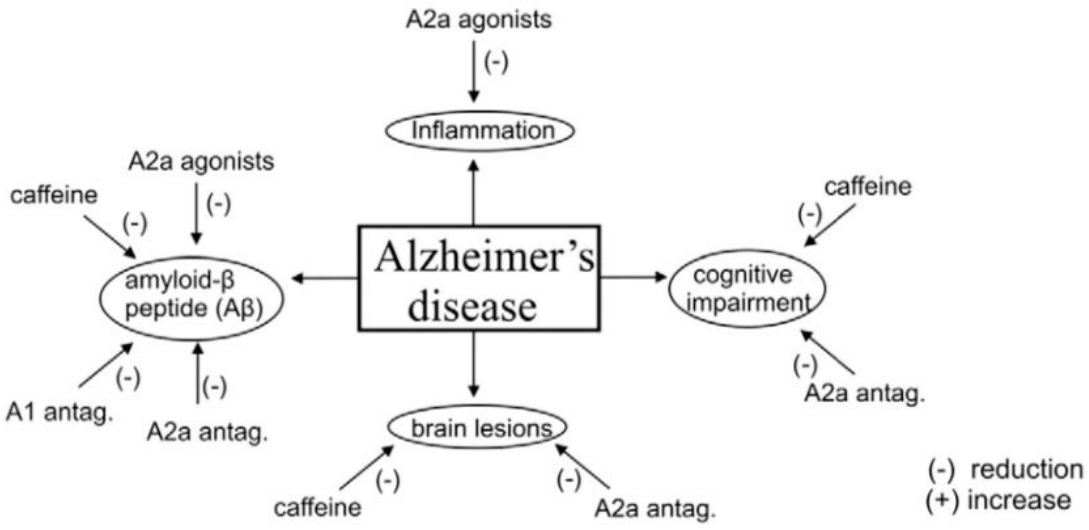

Fig. 7 Flowchart showing the effects of adenosine antagonists on the development of Alzheimer's disease. The - and + represent reductions and increases in the symptoms of the correlated diseases, respectfully. In Alzheimer's disease, adenosine antagonists and caffeine reduced brain lesions, cognitive impairments, inflammation, and amyloid-beta protein concentration (Rivera-Oliver & Diaz-Rioz, 2014).

Caffeine has been shown to reduce the amounts of amyloid-beta peptide in the brain, translating well to Alzheimer's disease because the disruptive plaques in Alzheimer's patients are made of amyloid-beta 1-42 and 1-40 (Panza et al., 2015; Rivera-Oliver & Diaz-Rioz, 2014). Researching potential ways to slow the progression and severity of the disease is crucial because the scope of Alzheimer's is increasing exponentially without a present cure, and its effects are far-reaching in both patients and indirectly affected citizens. By developing a treatment or cure, the mortality rate will decrease, as well as the large amounts of time and money spent annually caring for the diseased patients.

Purpose

The purpose of this study is to investigate a possible correlation between caffeine concentration in the brain and the presence of amyloid-beta proteins causing Alzheimer's disease. Amyloid-beta 1-42 and 1-40 are both factors in the pathology of Alzheimer's, so both types of protein are included in this investigation. Since caffeine has been shown to express various neuroprotective effects, especially the removal of toxic particles like amyloid-beta proteins, it is reasonable to consider whether caffeine has a role in preventing the disease development.

Research Question

Is there a relationship between caffeine concentration in the brain and amyloid-beta protein levels that are significant to late-onset Alzheimer's disease development?

Hypotheses

Null hypothesis: There is no significantly identifiable relationship between neural caffeine concentration and amyloid-beta protein levels.

Alternative hypothesis: There is a statistically significant relationship between neural caffeine concentration and amyloid-beta protein levels that indicates a negative correlation between caffeine concentration and amyloid-beta protein levels.

Methods

Literature & Information Search

To gather papers for this systematic literature review, the electronic bibliographic databases EBSCOhost, Google Scholar, PubMed, ResearchGate, Cochrane Library, JSTOR, and AAAS were used. Additional research papers from reference lists of articles obtained were also collected. All of the articles used were peer-reviewed, and they either provided background information useful for gaining a further understanding of current research on the topic, or they offered quantitative data based on conducted experiments The date range of the source publications ranged from 1980 to present to gain an understanding of research progress on Alzheimer's disease without including prior studies that may contain inaccurate information due to the level of technological and scientific resources at the time. However, this study focused solely on articles from 2000 to the present that contained quantitative data relevant to the project to maximize the accuracy and information available in current times. The source publications accessed were in English. Articles were found by using the search terms "caffeine and adenosine", "caffeine and amyloid-beta", "caffeine and Alzheimer's disease", and "caffeine and

neurobiology" in both electronic databases and scientific journals. Peer-reviewed papers concerning the molecular structure of caffeine, caffeine-adenosine antagonism, and caffeine and amyloid-beta relationships were read through and annotated, and reference lists and bibliographies of the papers were analyzed to conduct this review. Furthermore, scientists and authors who were experts in neurobiology with a focus in neurodegenerative disease and did lab experimentation concerning caffeine and amyloid-beta proteins were noted to potentially contact for information or access to other databases and articles.

Literature and Data Collection

Using the above search criteria to collect peer-reviewed articles, quantitative data was compiled from such relevant academic papers testing the effects of caffeine on the presence of amyloid-beta 1-40 and 1-42 proteins. The results from a number of articles were studied, recorded, and noted for further analysis. The control data was obtained from studies that included data on mice with no caffeine treatment and an average of controls for each amyloid-beta protein strain was taken to represent one data point. The experimental data consisted of primarily quantitative findings, backed up by qualitative trends. Quantitative data detailed amyloid-beta measurements following the treatments of mice cells with different dosages of caffeine. Supplementary qualitative data from peer-reviewed articles comprised of brain scan imaging visually marking the presence of amyloid-beta, comparing fluorescent imaging from a control of no caffeine with experimental caffeine treatment. The quantitative data in particular was amassed and interpreted through statistical tests, specifically a one-tailed t-test assuming unequal variance.

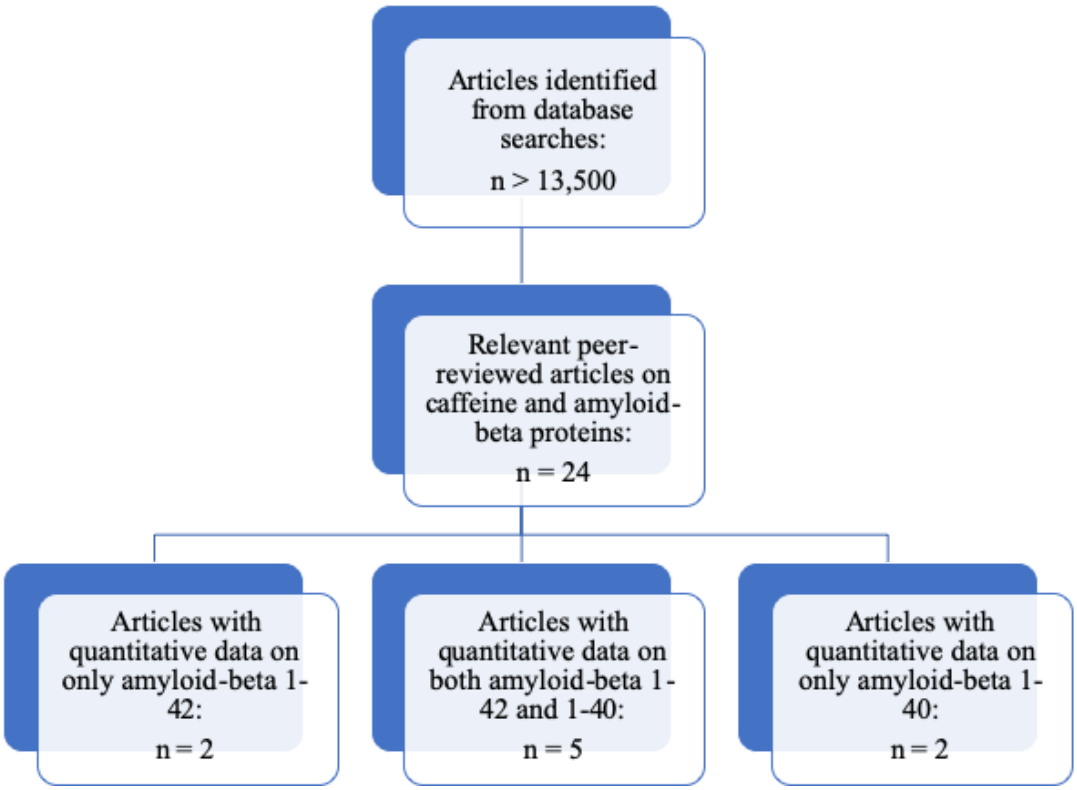

Fig. 8 Flowchart mapping the course of literature search using the specified criteria, starting with keyword searches and ending with peer-reviewed papers containing quantitative data.

In each of the peer-reviewed papers from which data were extracted, transgenic mice, mice whose brains were manipulated to incur late-onset Alzheimer's disease, were treated with different concentrations of caffeine through controlled water supplies. To gather experimental data, varying caffeine treatments resulting from caffeine's solution in water were prepared with differing concentrations, and noted and fed to the mice depending on their designation for testing particular concentrations. Control group mice were not administered any caffeine solution. Mice

brain cells were then used to determine the level of amyloid-beta protein following caffeine administration.

Only articles using transgenic mice for testing were included in this study to establish a baseline of disease consistency; using varied methods for inducing Alzheimer's in test subjects would affect data analysis because the presence of the disease would be influenced by a multitude of factors that may play a role in amyloid-beta production and persistence throughout neurons. Once symptoms of severe stage Alzheimer's were expressed, the mice brains were harvested and tested for amyloid-beta presence. The determined levels of amyloid-beta 1-40 and 1-42 following both treatment and control were then used to construct graphs, which the data points were extracted from to perform this study's synthesis and consequent statistical analysis.

Statistical Analysis

Following literature collection, compiled data from multiple peer-reviewed papers were analyzed using a one-tailed t-test assuming unequal variance and a linear regression for each type of amyloid-beta protein. A one-tailed t-test, performed on Microsoft's Analysis ToolPak, was chosen because the correlation being looked for was assumed to go only in one direction, that being a negative correlation between caffeine concentration and amyloid-beta levels. Unequal variances were assumed because the range of each data set, caffeine concentration and amyloid-beta levels, varied in magnitude. To interpret the t-test, a p-value of $p < 0.05$ was considered statistically significant, so if a p-value was under 0.05, the null hypothesis was rejected and alternative hypothesis accepted.

Additionally, a linear regression was also performed on the data to model the relationship between caffeine concentration and amyloid-beta levels visually. From a best fit line, an R-squared value was calculated, representing the variance of the data from the line. R-squared is measured out of 1.00, with such a value meaning a 100% fit with the linear model, so a calculated value of $R^2 > 0.65$ was interpreted as having an above average fit.

Results

Data Analysis

Out of the nine papers with quantitative data on the correlation between either amyloid-beta 1-42 or amyloid-beta 1-40, two had data on only amyloid-beta 1-42 and two had data on only amyloid-beta 1-40, with the rest having data on both proteins. Of these nine, two had qualitative data in the form of brain imaging showing the protein level difference between controlled transgenic mice and those treated with caffeine (Table 1).

Table 1. Characteristics of each peer-reviewed article used in the study, marked for having quantitative or qualitative data.

Study	**Quantitative AB 1-42 Data**	**Quantitative AB 1-40 Data**	**Qualitative Data**
Arendash, et al. (2006)	Y	Y	
Arendash, et al. (2009)	Y		
Arendash & Cao (2010)		Y	
Cao, et al. (2009)	Y	Y	
Chu, et al. (2012)	Y		Y
Dall'lgna, et al. (2003)	Y	Y	
Li, et al. (2015)	Y	Y	Y
Mancini, et al. (2018)		Y	
Zhang, et al. (2014)	Y	Y	

Within the seven papers that amyloid-beta 1-42 measurements were taken from, eight observed caffeine concentrations in units micromolarity (uM) were noted, each with a corresponding amyloid-beta level in picograms per milliliter (pg/mL). Within the seven papers that amyloid-beta 1-40 measurements were taken from, nine observed caffeine concentrations in units micromolarity were noted, each with a corresponding amyloid-beta level in picograms per milligram (pg/mg). For both sets of data, multiple amyloid-beta values were gathered for caffeine concentration zero, that being the control, and an average of these values were taken to

represent one data point. Both types of amyloid-beta experienced a decrease in concentration as the concentration of caffeine increased. Since amyloid-beta 1-42 and 1-40 were measured in different units, a comprehensive comparison between both was unable to be determined. However, each had significant declines in protein levels; there was an observed 98.4 pg/mL decrease of amyloid-beta 1-42 from 0 μM to 20 μM of caffeine, and a 1845 pg/mg decrease of amyloid-beta 1-40 from 0 μM to 63.3 μM of caffeine (Table 2; Table 3).

Table 2. Amyloid-beta 1-42 levels recorded at changing caffeine concentrations in a range from 0 to 20 μM.

Caffeine Concentration (μM)	**Amyloid-Beta 1-42 (pg/mL)**
0	109.97
0.1	120.0
0.25	99.0
0.5	90.0
5.15	79.0
10	49.0
16.51	21.5
20	11.62

Table 3. Amyloid-beta 1-40 levels recorded at changing caffeine concentrations in a range from 0 to 63.34 μM.

Caffeine Concentration (μM)	**Amyloid-Beta 1-40 (pg/mg)**
0	5646
0.1	7487.5
0.25	6062.5
0.5	5337.5
4.12	6550
5.97	6275
21.63	4650
57.98	4200
63.34	3800

After recording the data, a linear regression and a one-tailed t-test assuming unequal variance were performed on each set. The eight observed values in the amyloid-beta 1-42 data set were plotted on a scatter plot and a best fit line was constructed for the linear regression. The slope of the line was calculated to be -4.9287 and the R-squared value was 0.9549, or 95.49% linear fit (Figure 9). The following one-tailed t-test calculated a p-value of approximately 0.0014, which was below the critical value of 0.05, indicating a statistical significance (Table 4).

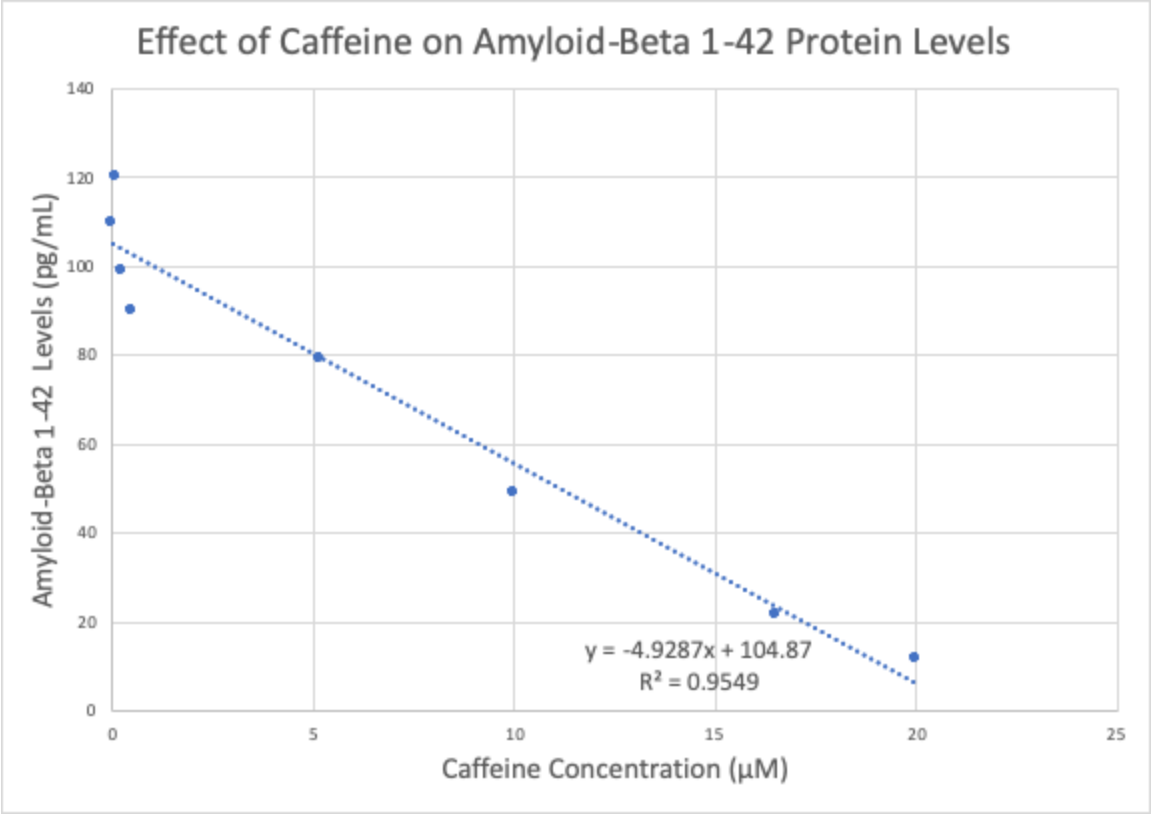

Fig. 9. Graph depicting amyloid-beta 1-42 levels as concentration increases and a best fit line determined from eight observed points.

Table 4. Synthesized data table showing a two-sample t-test assuming unequal variances. Variables are caffeine concentration measured in micromolarity and amyloid-beta 1-42 protein levels measured in picograms per milliliter of extracellular fluid in transgenic mice brains with induced late-onset Alzheimer's. Both one-tail and two-tail p-values provided.

	Caffeine Concentration (μM)	*Amyloid-Beta 1-42 (pg/mL)*
Mean	6.008	69.137115
Variance	69.25088343	1694.00832
Observations	8	8
Hypothesized Mean Difference	0	
df	8	
t Stat	-4.252228705	
P(T<=t) one-tail	0.001395344	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.002790688	
t Critical two-tail	2.306004135	

The nine observed values in the amyloid-beta 1-40 data set were also plotted and a best fit line constructed for the linear regression. The slope of the line was calculated to be -38.171 and the R-squared value was 0.6792, or a 67.92% linear fit (Figure 10). The one-tailed t-test calculated a p-value of approximately $3.3*10^{-7}$, indicating statistical significance (Table 5).

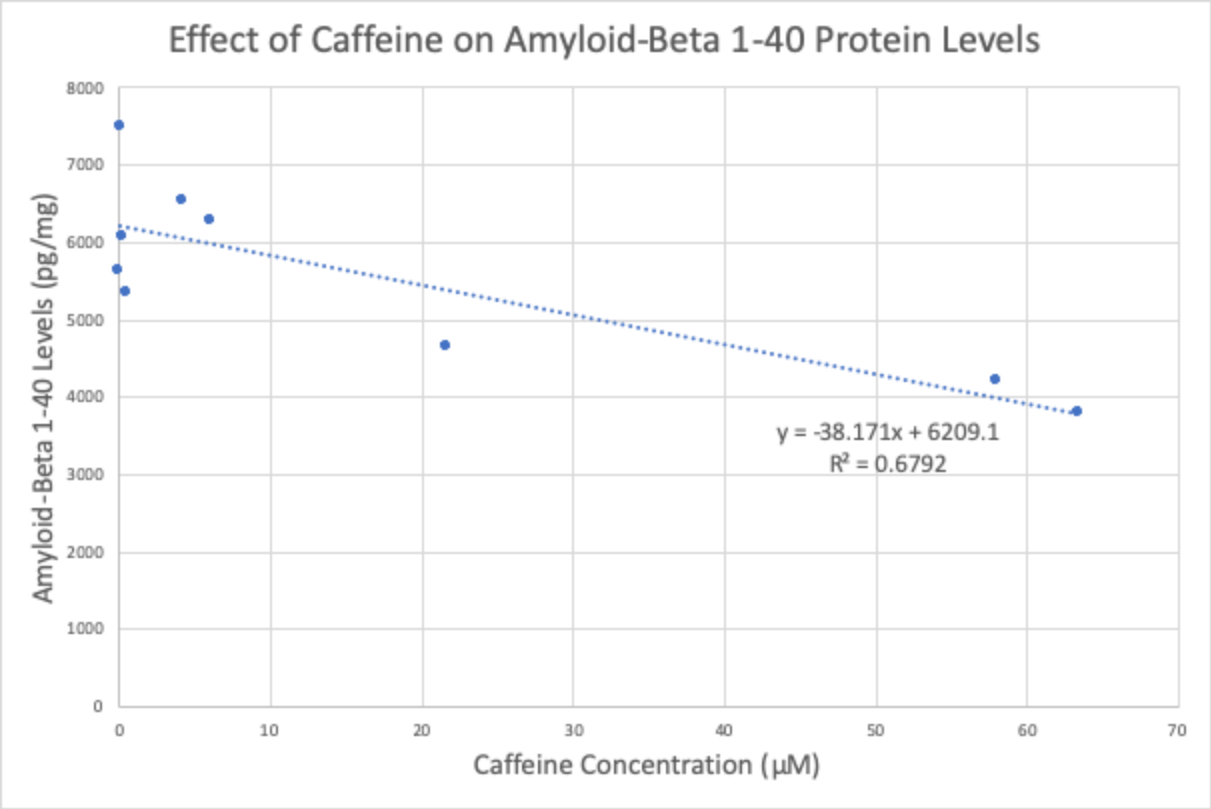

Fig. 10. Graph depicting amyloid-beta 1-40 levels as concentration increases and a best fit line determined from eight observed points.

Table 3. Synthesized data table showing a two-sample t-test assuming unequal variance. Variables are caffeine concentration measured in micromolarity and amyloid-beta 1-40 protein levels measured in picograms per milligram of extracellular fluid in transgenic mice brains with induced late-onset Alzheimer's. Both one-tailed and two-tailed p-values provided.

	Caffeine Concentration (uM)	*Amyloid-Beta 1-40 (pg/mg)*
Mean	17.09955399	5556.388889
Variance	657.9204254	1411375.174
Observations	9	9
Hypothesized Mean Difference	0	
df	8	
t Stat	-13.98469076	
P(T<=t) one-tail	3.31332E-07	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	6.62663E-07	
t Critical two-tail	2.306004135	

Following the data collection and extraction, it was noted that out of the nine papers analyzed, two contained cell imaging showing the effects of caffeine on the amyloid-beta proteins and the consequent cell response. This supplementary information was included in this study to further express quantitative findings through visual representations. Both papers indicated a decrease in amyloid-beta or related symptoms as a result of caffeine treatment, although through different aspects. Displayed images in Figure 11 utilized fluorescent

biomarkers that highlighted dead neurons in cultures of amyloid-beta presence with no caffeine treatment, and amyloid-beta presence with caffeine treatment (Figure 11; Chu et al., 2012). Images in Figure 12 used in vitro assays that detected amyloid-beta aggregation with administered caffeine solutions with concentrations ranging from 0 μM to 200 μM, and a control of no amyloid-beta presence (Figure 12; Li et al., 2015).

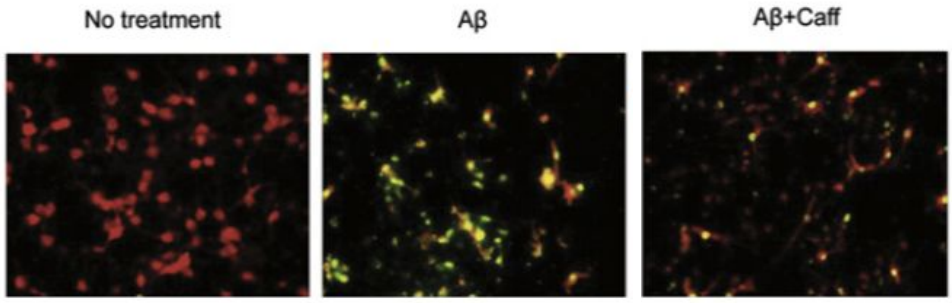

Fig. 11 Images of neurons with (Aβ+Caff) and without caffeine treatment (Aβ) compared to neurons with no amyloid-beta or caffeine. Dead neurons were marked in yellow and healthy neurons in red (Chu et al., 2012).

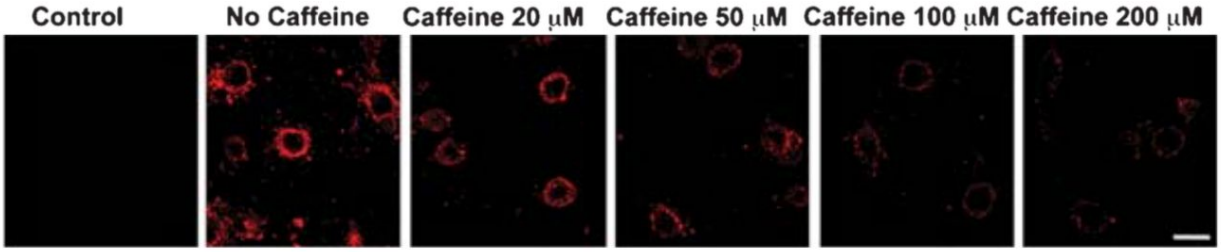

Fig. 12 Amyloid-beta aggregation, highlighted in red, in cells treated with increasing caffeine concentrations and compared to cells with no aggregation shown (Li et al., 2015).

Discussion

When analyzing both sets of amyloid-beta data, it is clear that increasing caffeine concentrations are correlated with decreasing amyloid-beta protein concentrations, as evident in Tables 2 and 3, where amyloid-beta 1-42 decreased by 98.4 pg/mL and amyloid-beta 1-40 decreased by 1845 pg/mg in the given caffeine concentrations. Both of these results support the alternative hypothesis, stating that a significant negative relationship between caffeine and amyloid-beta exists. Specifically, in data with amyloid-beta 1-42, the linear regression produced a trendline ($y=-4.9287x+104.87$) with an R-squared value of 0.9549, showing an overall decrease in amyloid-beta 1-42 with a 95.49% explanatory fit. The R-squared value, being close to 1.00, indicates a precise linear model that expresses the change in amyloid-beta 1-42 to increasing caffeine concentrations as having a negative correlation. Additionally, running a one-tailed t-test to determine the significance of the noticed protein reductions calculated a p-value of 0.001, below the accepted critical value of $p=0.05$, suggesting that the trend in data was statistically significant. This analysis of the amyloid-beta 1-42 data supports the alternative hypothesis that caffeine and amyloid-beta concentrations have a negative correlation, and rejects the null.

In data concerning amyloid-beta 1-40 proteins, the same linear regression and t-tests were performed to find R-squared and p-values. The linear regression model produced a trendline ($y=-38.171x+6209.1$) with an R-squared value of 0.6792, indicating a general decline in amyloid-beta 1-40 with a 67.92% fit. The relatively smaller R-squared in this model compared to the amyloid-beta 1-42 model is due to the scattered measurements from caffeine concentrations 0 to 6.0 μM, which were the farthest from the line of best fit. Even so, because the R-squared is

greater than the accepted value of 0.65, the linear model is accepted as being fairly accurate, just not very precise. In spite of a lower R-squared value, the t-test calculated a p-value of $3.3*10^{-7}$, which is smaller than that of the amyloid-beta 1-42 model. Since the p-value is below the accepted p=0.05, the relationship mapped by the data is statistically significant and supports the alternative hypothesis.

Although not statistically proven, the supplementary images support the general findings of this study. Visually, there is a noticeable difference in both amyloid-beta aggregation and amyloid-beta-caused neuron death as caffeine treatments were administered. The amount of dead neurons was significantly less in the image showing cells treated with caffeine as compared to the image showing no treatment (Figure 11). Additionally, the amount of amyloid-beta aggregation lessened in consecutive images of cells with caffeine treatments of increasing concentration. Cells with no caffeine showed distinct aggregation, whereas cells with 20 μM caffeine showed little to no aggregation (Figure 12). It is important to note, however, that in both image series, amyloid-beta presence and resulting symptoms were not completely removed; there are both signs of neuron death and aggregation highlighted in each figure (Figure 11; Figure 12). This is because it is natural for cell death and amyloid-beta to be apparent in the brain, but much larger magnitudes of these characteristics are expressed in Alzheimer's disease. Even so, the qualitative data provides support for the alternative hypothesis without proving it true.

These findings suggest that the presence of caffeine in the brain does lower amyloid-beta 1-42 and 1-40 levels, and that subsequently, caffeine may slow the progression of late-onset Alzheimer's disease through its effects on the proteins.

Conclusion

Based on the synthesis of results and statistical analysis thereof, a negative correlation between concentrations of Alzheimer's-inducing proteins and concentrations of caffeine is suggested. All the data collected expresses a general negative relationship between the two, with the concentration of proteins decreasing as the concentration of caffeine increases. As a result, the null hypothesis stating that caffeine does not affect Alzheimer's through amyloid-beta aggregation was rejected, and the alternative hypothesis was accepted, meaning that there is a statistically significant relationship between caffeine concentration and amyloid-beta protein levels where an increase in caffeine concentration leads to a decrease in amyloid-beta proteins.

Sources of Error

Since the methods in this study consisted of a systematic literature review and statistical analysis, none of the data collected came from a personally controlled study. Within the resulting data collection, some sources of error became apparent. All peer-reviewed studies from which data were extracted used transgenic mice to induce Alzheimer's disease within their brains. However, the species and genders of mice differed between papers, potentially affecting the susceptibility of the mice to Alzheimer's and how much amyloid-beta protein was produced in each, since clinical research has previously shown genetic markers to be a factor in the prevalence and severity of the disease. Additionally, the quantitative data extracted from the papers used came from graphical approximation, as data points were plotted on graphs and no supplementary raw data was made available. As a result, specific data points were approximated

and may not be precisely accurate. Caffeine treatment derivatives were also a possible source of error, as one study utilized a 95% pure caffeine treatment by extracting caffeine from ground coffee, which may have contained residual nutrients or molecules affecting the presence of amyloid-beta, whether inhibiting or promoting the protein growth (Chu, et al., 2012). However, since the collected data showed a cohesive correlation, the variability in data point accuracy did not take away from the general relationship between caffeine concentration and amyloid-beta levels, but rather the accuracy of the specific statistical values.

Further Work

With the correlation suggested in the data above, it would be informative to conduct independent lab work to gather further quantitative data. Because this study solely included work done on transgenic mice, conducting research on human cell lines or performing clinical trials would be beneficial to translate findings to a human audience. Concrete evidence arisen from multiple experimental tests ensuring accuracy would reinforce a relationship and increase the reliability of results. Additionally, models and simulations could be constructed to illustrate the effects of caffeine antagonism on the amyloid-beta proteins themselves to further visualize this phenomenon at the cellular level. The data extracted from these peer-reviewed academic works can also be used to fuel research in novel drug pathway therapies to treat late-onset Alzheimer's disease and other similar neurodegenerative diseases. Crystallizing caffeine extract to potentially slow the growth of amyloid-beta plaques in late-onset patients may pave way for a new and effective noninvasive treatment. The quantitative numbers may also be applied to adenosine

antagonized brain disorders, specifically other dementias, Parkinson's disease, or Huntington's disease, as similarities have been found in the relation of antagonized-adenosine receptor caused neurodegenerative diseases with other diseases of the same cause.

Conflict of Interest

In Chu et al.'s paper, it was disclosed that there two of the scientists working on the study were employees of Kraft Foods Global Brands LLC, which produced the coffee grounds they directly treated brain cells with by extracting caffeine to make cell treatments (Chu et al., 2012). However, coupled with the other research papers analyzed in this systematic literature review, the results should not be biased as other similar correlations are shown in the majority of the research.

Acknowledgments

I would like to thank my professional mentors, Dr. Maryann Martone, recall professor in the Department of Neurosciences at the University of California San Diego and executive director of FORCE11, and Dr. Vahri Beaumont, director of neurobiology at CHDI Foundation and past senior researcher at Merck. The help given advising data collection, writing, and giving advice within the neurobiology field was greatly appreciated. Additionally, thank you to my research advisor and internal mentor, Dr. Nikki Malhotra, and to Thousand Oaks High School and the Center for Advanced Studies and Research for giving the opportunity to conduct my independent research.

References

6 health ailments that accompany Alzheimer's. (2017). Retrieved from https://www.alzheimers.net/alzheimers-health-ailments/

Adenosine. (n.d.). Retrieved from https://www.webmd.com/vitamins/ai/ingredientmono-1067/adenosine

Allied Academies. (2019). [Graphic comparison of healthy vs. Alzheimer's brain and neurons]. *13th International Conference on Alzheimer's Disease and Dementia.* Retrieved from http://alzheimers.alliedacademies.com/events-list/http-alzheimers-alliedacademies-com

Alzheimer's Association. (2019). [Infographic of facts and figures pertaining to Alzheimer's disease]. *Alzheimer's Disease Facts and Figures*. Retrieved from https://www.alz.org/alzheimers-dementia/facts-figures

Alzheimer's Society. (2019). Who gets Alzheimer's disease? [Webpage]. Retrieved from https://www.alzheimers.org.uk/about-dementia/types-dementia/who-gets-alzheimers-disease#content-start

American Health Assistance Foundation. (2004). [Graphic comparison of normal and Alzheimer's neurons]. *About Amyloid Plaques*. Retrieved from http://www.ahaf.org/alzdis/about/AmyloidPlaques.htm

Amyloid beta precursor protein. (2019). Retrieved from https://ghr.nlm.nih.gov/gene/APP

Arendash, G. W., Mori, T., Cao, C., Mamcarz, M., Runfeldt, M., Dickson, A.,...Potter, H. (2009). Caffeine reverses cognitive impairment and decreases brain amyloid-β levels in aged Alzheimer's disease mice. *Journal of Alzheimer's Disease, 17*(3), 661-680. doi: 10.3233/JAD-2009-1087

Arendash, G. W., Schleif, W., Rezai-Zadeh, K., Jackson, E. K., Zacharia, L. C., Cracchiolo, J. R.,...Tan, J. (2006). Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain β-amyloid production. *Neuroscience, 142,* 941-952. doi:10.1016/j.neuroscience.2006.07.021

Arendash, G. W. & Cao, C. (2010). Caffeine and coffee as therapeutics against Alzheimer's disease. *Journal of Alzheimer's Disease, 20*, 117-126. DOI 10.3233/JAD-2010-091249

Bello, I. & Salerno, M. (2014).Evidence against a role of P-glycoprotein in the clearance of the Alzheimer's disease $A\beta_{1-42}$ peptides. *Cell Stress and Chaperones, 20*(3), 421-430. doi: 10.1007/s12192-014-0566-8.

Bright Focus Foundation. (2017). *The Progression of Alzheimer's Disease*. Retrieved from https://www.brightfocus.org/alzheimers/infographic/progression-alzheimers-disease

Cao, C., Cirrito, J. R., Lin, X., Wang, L., Verges, D. K., Dickson, A.,...Potter, H. (2009). Caffeine suppresses amyloid-β levels in plasma and brain of Alzheimer's disease transgenic mice. *Journal of Alzheimer's Disease, 17,* 681-697. DOI 10.3233/JAD-2009-1071

Cao, R., Rossetti, G., Bauer, A., & Carloni, P. (2015). Binding of the antagonist caffeine to the human adenosine receptor hA2AR in nearly physiological conditions. *PLoS ONE, 10*(5). Retrieved from https://doi.org/10.1371/journal.pone.0126833

Carter, J. & Lippa, C. F. (2001). Beta-amyloid, neuronal death and Alzheimer's disease. *Current Molecular Medicine, 1*(6), 733-737. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11899259

Chen, J. F., Lee, C. F., & Chern, Y. (2014). Adenosine receptor neurobiology: Overview.

International Review of Neurobiology, 119, 1-49. doi: 10.1016/B978-0-12-801022-8.00001-5.

Chu, Y., Chang, W., Black, R. M., Liu, J., Sompol, P., Chen, Y.,...Cheng, I. H. (2012). Crude caffeine reduces memory impairment and amyloid β_{1-42} levels in an Alzheimer's mouse model. *Food Chemistry, 135,* 2095-2102. http://dx.doi.org/10.1016/j.foodchem.2012.04.148

Colombo, R. & Papetti, A. (2019). An outlook on the role of decaffeinated coffee in neurodegenerative diseases. *Critical Reviews in Food Science and Nutrition.* https://doi.org/10.1080/10408398.2018.1550384

Costa, M. S., Botton, P. H., Mioranzza, S., Souza, D. O., & Porciuncula, L. O. (2008). Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tirosine kinase receptor (TrkB) content in mice. *Neuroscience, 153*, 1071-1078. doi:10.1016/j.neuroscience.2008.03.038

Dall'Igna, O. P., Fett, P., Gomes, M. W., Souza, D. O., Cunha, R. A., & Lara, D. R. (2007). Caffeine and adenosine A_{2a} receptor antagonists prevent β-amyloid (25-35)-induced cognitive deficits in mice. *Experimental Neurology, 203,* 241-245. doi:10.1016/j.expneurol.2006.08.008

Dall'lgna, O. P., Porciuncula, L. O., Souza, D. O., Cunha, R. A., & Lara, D. R. (2003). Neuroprotection by caffeine and adenosine A_{2a} receptor blockade of β-amyloid neurotoxicity. *British Journal of Pharmacology, 138,* 1207-1209. doi:10.1038/sj.bjp.0705185

Deguo, D., Murray, A. N., Cohen, E., Kim, H., Simkovsky, R., Dillin, A., & Kelly, J. W. (2011).

A kinetic aggregation assay enabling selective and sensitive Aβ amyloid quantification cells and tissues. *Biochemistry, 50*(10), 1607-1617. doi: 10.1021/bi1013744

Dhinesh, K. M. (2018). [Diagram comparing the molecular structures of caffeine and adenosine]. *FossCrunch Explainer*. Retrieved from https://fosscrunch.com/what-does-caffeine-do-to-your-brain/

Drolle, E., Hane, F. T., Lee, B., & Leonenko, Z. (2014). Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *Drug Metabolism Reviews, 46*(2). DOI: 10.3109/03602532.2014.882354

Eskelinen, M. H. & Kivipelto, M. (2010). Caffeine as a protective factor in dementia and Alzheimer's disease. *Journal of Alzheimer's Disease, 20*(1), 167-174. doi: 10.3233/JAD-2010-1404.

Fredholm, B. B. (1995). Adenosine, adenosine receptors and the actions of caffeine. *Pharmacology & Toxicology, 76*(2). https://doi.org/10.1111/j.1600-0773.1995.tb00111.x

Grippo, R. M., Purohit, A. M., Zhang, Q., Zweifel, L. S., & Güler, A. D. (2017). Direct midbrain dopamine input to the suprachiasmatic nucleus accelerates circadian entrainment. *Current Biology, 27,* 2465-2475. http://dx.doi.org/10.1016/j.cub.2017.06.084

Hardy, J. & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science's Compass, 297*(5580), 353-356. doi:10.1126/science.1072994

Hardy, J. A. & Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science, 256*(5054). Retrieved from https://go.galegroup.com/ps/anonymous?id=GALE%7CA12207965&sid=googleScholar

&v=2.1&it=r&linkaccess=abs&issn=00368075&p=HRCA&sw=w

Hu, K., Van Someren, E. J. W., Shea, S. A., Scheer, F. A. J. L., & Stanley, H. E. (2009). Reduction of scale invariance of activity fluctuations with aging and Alzheimer's disease: Involvement of the circadian pacemaker. *Proceedings of the National Academy of Sciences of the United States of America, 106*(8), 2490-2494. www.pnas.org/cgi/doi/10.1073/pnas.080608

Institute for Scientific Information on Coffee. (2019). [Diagram of caffeine antagonism on adenosine]. *Caffeine and Performance in Endurance (Aerobic) Exercise.* Retrieved from https://www.coffeeandhealth.org/topic-overview/caffeine-and-performance-in-endurance-aerobic-exercise/caffeine-binding-to-the-adenosine-receptor/

Isik, A. T. (2010). Late onset Alzheimer's disease in older people. *Clinical Interventions in Aging, 5,* 307-311. doi: 10.2147/CIA.S11718

Johns Hopkins Medicine. (2019). Stages of Alzheimer's Disease [Webpage]. Retrieved from https://www.hopkinsmedicine.org/health/conditions-and-diseases/alzheimers-disease/stages-of-alzheimer-disease

Khasnabish, S. (2015, November 11). The paradox of cell death [web log post]. Retrieved from https://sites.bu.edu/ombs/category/alzheimers-disease/

Kim, T., Vidal, G. S., Djurisic, M., William, C. M., Birnbaum, M. E., Garcia, K. C.,...Shatz, C. J. (2013). Human LilrB2 is a β-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. *Science Mag, 341*(6152), 1399-1404. DOI: 10.1126/science.1242077

Koedam, E. L., Lauffer, V., van der Viles, A. E., van der Flier, W. M., Scheltens, P., &

Pijnenburg, Y. A. (2010). Early-versus late-onset Alzheimer's disease: More than age alone. *Journal of Alzheimer's Disease, 19*(4), 1401-1408. doi: 10.3233/JAD-2010-1337.

Kolahdouzan, M. & Hamadeh, M. J. (2017). The neuroprotective effects of caffeine in neurodegenerative diseases. *CNS Neuroscience & Therapeutics, 23*(4), 272-290. doi: 10.1111/cns.12684.

Li, J. Z., Bunney, B. G., Meng, F., Hagenauer, M. H., Walsh, D. M., Vawter, M. P.,...Bunney, W. E. (2013). Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences of the United States of America, 110*(24), 9950-9955. http://www.pnas.org/cgi/doi/10.1073/pnas.1305814110

Li, S., Geiger, N. H., Soliman, M. L., Hui, L., Geiger, J. D., & Chen, X. (2015). Caffeine, through adenosine A_3 receptor-mediated actions, suppresses amyloid-β protein precursor internalization and amyloid-β generation. *Journal of Alzheimer's Disease, 47*(1), 73-83. doi:10.3233/jad-142223

Mancini, R. S., Wang, Y., & Weaver, D. F. (2018). Phenylindanes in brewed coffee inhibit amyloid-beta and tau aggregation. *Frontiers in Neuroscience, 12*(735). doi: 10.3389/fnins.2018.00735

Martins, I. J. (2016). Food intake and caffeine determine amyloid beta metabolism with relevance to mitophagy in brain aging and chronic disease. *European Journal of Food Science and Technology, 4*(5), 11-17. Retrieved from https://ro.ecu.edu.au/ecuworkspost2013/3421

Meyer, A., Siethoff, C., Sucharski, F., & Orth, M. (2017). [Three-dimensional model of an

amyloid-beta peptide]. *Quantification of Amyloid Beta Peptides in Human Cerebrospinal Fluid USing Micro HPLC-HRMS.* Retrieved from https://www.prolab.ch/application-note-quantification-amyloid-beta-peptides-human-cerebrospinal-fluid-using-micro-hplc-hrms/

Mohan, A., Roberto, A. J., Mohan, A., Liogier-Weyback, L., Guha, R., Ravishankar, N.,...Mohan, R. (2015). Caffeine as a treatment for Alzheimer's Disease: A review. *Journal of Caffeine Research, 5*(2). DOI: 10.1089/jcr.2014.0027

Moreira, P. I., Nunomura, A., Honda, K., Aliev, G., Casadesus, G., Zhu, X.,...Perry, G. (2007). The key role of oxidative stress in Alzheimer's disease. In *Oxidative stress and neurodegenerative disorders* (12). https://doi.org/10.1016/B978-044452809-4/50153-8

Murphy, M. P. & LeVine III, H. (2010). Alzheimer's disease and the β-amyloid peptide. *Journal of Alzheimer's Disease, 19*(1), 311. doi:10.3233/JAD-2010-1221.

National Institutes of Health. (2016). What happens to the brain in Alzheimer's disease? [Webpage]. Retrieved from https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease

Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A., & Feeley, M. (2010). Effects of caffeine on human health. *Food Additives & Contaminants, 20*(1), 1-30. https://doi.org/10.1080/02652030210000078 40

Noble, W., Hanger, D. P., Miller, C. C. J., & Lovestone, S. (2013). The importance of tau phosphorylation for neurodegenerative diseases. *Frontiers in Neurology, 4*(83). doi: 10.3389/fneur.2013.00083

Panza, F., Solfrizzi, V., Barulli, M. R., Bonfiglio, C., Guerra, V., Osella, A.,...Logroscino, G. (2015). Coffee, tea, and caffeine consumption and prevention of late-life cognitive decline and dementia: A systematic review. *Journal of Nutrition, Health, and Aging, 19*(3), 313-328. doi: 10.1007/s12603-014-0563-8.

Peterson, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J. C., Rabins, P. V.,...Winblad, B. (2001). Current concepts in mild cognitive impairment. *JAMA Neurology, 58*(12), 1985-1992. doi:10.1001/archneur.58.12.1985

Qiu, T., Liu, Q., Chen, Y. X., Zhao, Y. F., & Li, Y. M. (2015). Aβ42 and Aβ40: similarities and differences. *Journal of Peptide Science, 21*(7), 522-529. doi: 10.1002/psc.2789

Rahman, A. (2009). The role of adenosine in Alzheimer's disease. *Current Neuropharmacology, 7*(3), 207-216. doi: 10.2174/157015909789152119

Ribeiro, J. A. & Sebastião, A. M. (2010). Caffeine and adenosine. *Journal of Alzheimer's Disease, 20*(1), 3-15. doi: 10.3233/JAD-2010-1379.

Ritchie, K., Carriere, I., de Mendonca, A., Portet, F., Dartigues, J. F., Rouaud, P.,...Ancelin, M. L. (2007). The neuroprotective effects of caffeine: A prospective population study (the Three City Study). *Neurology, 69*(6), 536-545. https://doi.org/10.1212/01.wnl.0000266670.35219.0c

Rivera-Oliver, M. & Díaz-Ríos, M. (2014). Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: A review. *Life Sciences, 101*(0), 1-9. doi: 10.1016/j.lfs.2014.01.083

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron, 6,* 487-498. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1673054

Selkoe, D. J. (2001). Alzheimer's disease: Genes, proteins, and therapy. *Physiological Reviews, 81*(2), 741-766. DOI: 10.1152/physrev.2001.81.2.741

Sharma, B., Kalita, S., Paul, A., Mandal, B., & Paul, S. (2016). The role of caffeine as an inhibitor in the aggregation of amyloid forming peptides: a unified molecular dynamics simulation and experimental study. *RSC Advances*, *6*(82), 78548-78558. 10.1039/C6RA17602J

Skoog, I. (2014). Is it dangerous or beneficial to drink coffee? Reflections on a meta-analysis on risk at birth and a population study on risk in late life. *European Journal of Epidemiology, 29,* 665-666. DOI 10.1007/s10654-014-9956-6

Slats, D., Claassen, J. A. H. R., Verbeek, M. M., & Overeem, S. (2013). Reciprocal interactions between sleep, circadian rhythms, and Alzheimer's disease: Focus on the role of hypocretin and melatonin. *Ageing Research Reviews, 12,* 188-200. http://dx.doi.org/10.1016/j.arr.2012.04.003

Tarasoff-Conway, J. M., Carare, R. O., Osorio, R. S., Glodzik, L., Butler, T., Fieremans, E.,...de Leon, M. J. (2015). Clearance systems in the brain--Implications for Alzheimer disease. *Nature Reviews Neurology, 11*(8), 457-470. doi: 10.1038/nrneurol.2015.119

Tate, B., Aboody-Guterman, K. S., Morris, A. M., Walcott, E. C., Majocha, R. E., & Marotta, C. A. (1992). Disruption of circadian regulation by brain grafts that overexpress Alzheimer β/A4 amyloid. *Proceedings of the National Academy of Sciences of the United States of America, 89*(15), 7090-7094. Retrieved from https://www.jstor.org/stable/2359937

Very Well Health. (2018). Signs of preclinical Alzheimer's disease [Webpage]. Retrieved from https://www.verywellhealth.com/what-are-the-signs-of-preclinical-alzheimers-disease-98

593

Yenisetti, S. C. & Muralidhara, M. (2016). Beneficial role of coffee and caffeine in neurodegenerative diseases: A minireview. *AIMS Public Health, 3*(2), 407-422. DOI: 10.3934/publichealth.2016.2.407

Zhang, L., Zhou, Z., Wang, Z., Du, Y., He, Z., Cao, C., & Zhou, S. (2014). Coffee and caffeine potentiate the antiamyloidogenic activity of melatonin via inhibition of Aβ oligomerization and modulation of the Tau-mediated pathway in N2a/APP cells. *Drug Design, Development, and Therapy, 9,* 241-272. http://dx.doi.org/10.2147/DDDT.S71106

Zheng, H. & Koo, E. H. (2011). Biology and pathophysiology of the amyloid precursor protein. *Molecular Neurodegeneration, 6*(27). https://doi.org/10.1186/1750-1326-6-27