**Investigating the effect of knockout APP and increased Calcium levels in the aggregate formation and synaptic plasticity in Alzheimer’s disease**

**I. Introduction**

Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder that affects the memory and brain function of middle to old aged individuals4. The increasing prevalence of AD has caused major concern due to the fact that AD is a complex disorder that is difficult to diagnose4. Currently, the only accurate method to diagnose an individual with AD is to identify the pathological markers in post-mortem exams4. Without a complete understanding of the disorder, no cures have been developed to effectively treat AD. In order to develop treatments for individuals with AD, research has focused on investigating two hallmark characteristics associated with the disease: neurofibrillary tangles (NFT) and senile plaques (SP). NFTs are tangles that are composed of modified tau proteins, while SPs contain an aggregate form of the β-amyloid peptide6. Both NFTs and SPs are observed in individuals diagnosed with AD in postmortem studies and are responsible for the deterioration in brain function seen in individuals with AD4. Previous research suggests that these two proteins interact with each other directly4. An increase in the β-amyloid peptide causes an increase in the Tau protein levels4. This increase in Tau protein levels causes a build up of the protein in the extracellular matrix of neurons and leads to the formation of NFTs6.

As these are two of the common characteristics of AD, research has focused on reducing the presence of NFTs and SPs in order to slow the progression of the disease and improve brain function. Since the presence of the β-amyloid peptide is responsible for the formation of NFTs, decreasing or eliminating the β-amyloid peptide would reduce the presence of NFTs in the brain, thereby slowing the progression of AD in the brain.

Research has focused on the origin of the β-amyloid peptide in order to decrease β-amyloid peptide levels and hinder the formation of NFTs in the brain. The APP gene encodes for the amyloid precursor protein1. This protein is cut into peptides by enzymes known as secretases1. One of these peptide fragments is the β-amyloid peptide1. The β-amyloid peptide is likely to be involved in the plasticity of neurons, one of the functions that are impaired in individuals with AD1. Since the β-amyloid peptide is synthesized from the APP gene, silencing this gene will prevent the synthesis of the β-amyloid peptide. This will, in turn, prevent the formation of NFTs and SPs, slowing down the progression of AD in individuals. However, research has shown that silencing the APP gene decreases the synaptic plasticity of neurons by decreasing the release of synaptic protein vesicles from the presynaptic neurons in the brain3.

Increasing intracellular calcium levels in neurons have been shown to increase synaptic plasticity8. One method to increase intracellular calcium levels is to open the NMDA channels within the neurons2. NMDA channels can be opened by the neurotransmitter glutamate9. Thus, incubating neurons in a media containing glutamate can open NMDA channels; thereby increasing the intracellular calcium levels. An increase in calcium levels activates the CaMKII kinase8. This kinase increases long-term potentiation and enhances the efficacy of synaptic transmission8. Abnormal levels of CaMKII have also been shown to be involved in AD8. Thus, this proposal aims to design an experiment that tests whether increasing intracellular calcium levels through exogenous methods will counteract the effects that silencing APP has on synaptic plasticity.

**II. Experiment**

The purpose of this experiment is to determine a method in which the ability of presynaptic neurons to secrete presynaptic vesicles containing neurotransmitters is not compromised when the APP gene is knocked out. If the presynaptic ability of neurons remains at a level similar to a control group, then an effective treatment for individuals with Alzheimer’s can be developed.

In order to conduct this experiment, hippocampal slices from mice diagnosed with Alzheimer’s disease should be isolated and prepared in a similar fashion to Gardoni et al. (2001). The researchers used slice electrophysiology, a technique in which specific regions of the brain are isolated and placed in an artificial cerebrospinal fluid in order to achieve greater experimental control by eliminating extraneous factors that can affect the synaptic plasticity of these neurons2. These cells then will be split up into three different groups. One group will serve as the control group in which the APP gene is not silenced. The second group of cells will have the APP gene silenced but no mechanism to increase presynaptic plasticity. The third group of cells will have the APP gene silenced and be provided with glutamate since glutamate activates NMDA receptors9. The third group is grown in a medium containing glutamate while the other two groups will be grown in a regular cell culture media.

Once these cells are isolated and sorted into their respective groups, silencing RNA (siRNA) molecules can be used to target the APP gene and silence the gene. To check if the APP gene was successfully silenced, the cells can be checked for the presence of NFTs. Since the APP gene codes for the β-amyloid peptide which cause the formation of NFTs by increasing Tau protein levels, the lack of NFTs indicates that the APP gene has been successfully silenced. Electron microscopy can be used to view the presence of NFTs. Electron microscopy allows researchers to observe small biological specimens5.

In order to determine if the presence of glutamate affected the synaptic plasticity of the hippocampal cells, the excitatory postsynaptic potential (ESPS) of the AMPA receptors should be measured7. The ESPS of these receptors is a measure of the synaptic plasticity of the neurons because it measures the potential of postsynaptic neurons7. The synaptic plasticity of the cells in each of the three groups should be measured in order to compare the levels of the synaptic plasticity between the three groups.

**III. Discussion**

The ideal results from this experiment include successfully silencing of the APP gene from the isolated hippocampal cells and then increasing the synaptic plasticity of these cells to normal levels. If the APP gene is silenced, then the β-amyloid peptide cannot be synthesized from the APP gene. This will in turn not cause an increase in the Tau protein levels and thus, prevent the formation of NFTs. Without the presence of NFTs and increased synaptic plasticity, the progression of AD will slow down in individuals. These results can then be used to develop effective treatments for individuals with AD to postpone or prevent the onset of the disease.

However, there is a possibility that the synaptic ability of the hippocampal cells may be compromised. The increase in calcium levels may not be enough to counteract the effect that the silencing of the APP gene has. If so, then the presynaptic neurons in the hippocampus will not be able to secrete the presynaptic vesicles. This will lead to decreased synaptic plasticity and eventually cell death.

In the case that synaptic plasticity is compromised even with increased calcium levels, then other methods to increase synaptic plasticity can be used or combined with the increase in calcium levels. Additionally, focusing specifically on certain areas that are known to be impaired in AD might be beneficial. Silencing the APP gene in only those specific regions of the brain may lower the amount of NFTs that are present in the brain without fully compromising the neuronal organ’s synaptic plasticity.

Regardless, mechanisms that will prevent the formation of NFTs and SPs show the most promise for future research into AD. Special focus should be placed on the APP gene and its related proteins as this gene is the source for the formation of NFTs and SPs. Extensive research into the various mechanisms involved in AD will hopefully lead to a treatment for individuals suffering from this neurodegenerative disease.

**References**

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