Synapse Distribution in a 5xFAD Mouse Model Using Immunolabeling and Tissue Clearing Techniques

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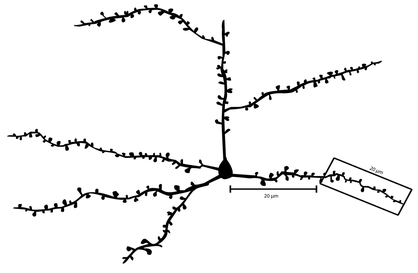
Alzheimer`s Disease (AD) is a progressive degenerative brain disease resulting in dementia and memory impairments in patients. Research is immensely important as there are currently no treatments available to slow, prevent, or cure the damage inflicted upon individuals. Our lab has previously demonstrated that Lipopolysaccharide (LPS) injections over seven days in mice induce an inflammatory response. Inflammation results in the formation of Amyloid- Beta (AB); prior research has shown that AB is found in high amounts in Alzheimer's patients. Studies postulate AB results in cognitive dysfunction by disrupting synaptic signaling. Synapses have been correlated with memory impairment. A synapse is associated with information transfer inside the brain; composed of pre-synaptic neurons, post-synaptic neurons, and additional supportive cells including glial cells and astrocytes. Neuroscientists are trying to determine if the synapse disruption due to Aβ leads to the cognitive dysfunction seen in AD. However, first scientists must see how Aβ results in the destabilization of neurons and synapses in mice. The problem is how difficult it is to visualize individual neurons inside the hippocampus. To study neuronal synapses, the present study will use an immunolabeling and tissue clearing technique in an AD transgenic mouse model. Antibodies will label GFP, Aβ, and a post synaptic neuron marker, PSD-95. We expect the tissue clearing technique which will clear lipids from the brain, to result in enhanced visualization of neurons and axon guidance defects in the brain. The study will see if the increased amount of Aβ in the hippocampus of GFP^{+}/FAD $^{+}$ mice results in a change in the number of dendritic spines compared to GFP^{+}/FAD^{-} mice. It is hypothesized that in comparison, the FAD+ mice will have fewer dendritic spines corresponding to the elevated levels of Aβ in their hippocampus.

Introduction

- Alzheimer's disease (AD) is a neurodegenerative disorder that affects over 5 million Americans, and there is currently no cure (1).
- AD is characterized by Aβ plaques, neurofibrillary tangles, inflammation, and neuronal/synapse loss in the brain (2).
- Our lab has shown that 7 consecutive days of LPS injections results in an increase in hippocampal expression of Aβ along with deficits in learning and memory (3).
- Oligomeric Aβ has been hypothesized to bind to receptors in neuronal synapses thus disrupting synapse signaling (4).
- A synapse is a gap between two neurons in which a signal passes through.
- We are trying to determine if Aβ is responsible for the decrease in synaptic density in the CA1 region of the hippocampus.
- This study will utilize a tissue clearing technique which will remove lipids from the brain and eliminate light scatter due to these fatty acids (5).
- We expect the tissue clearing technique to result in enhanced resolution of the images of the neurons and dendritic spines.
- Our hypothesis is that the FAD+ mice will have a decreased number of dendritic spines in comparison to FAD- mice.

Methods



- 4-5 month-old mice were chosen from either GFP+/FAD- or GFP+/FAD+ groups
- Brain tissue was processed using either a conventional staining technique or a tissue clearing technique as outlined in IDISCO+ protocol (6).
- Brains were sliced into 50 micron thin sections and synapses counted using confocal microscopic examination by volunteers blinded to the genotype.
- 3 brain sections were taken from each of the 8 mice chosen.
- For each section, dendritic spines were counted in a 20 micrometer section on the dendrites of 5 separate neurons in the CA1 region of the hippocampus.
- Dendritic spines must be .05 µm in height in order to be counted
- A t-test was conducted to determine statistical significance between FAD- and FAD+ groups.

Funding

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Results

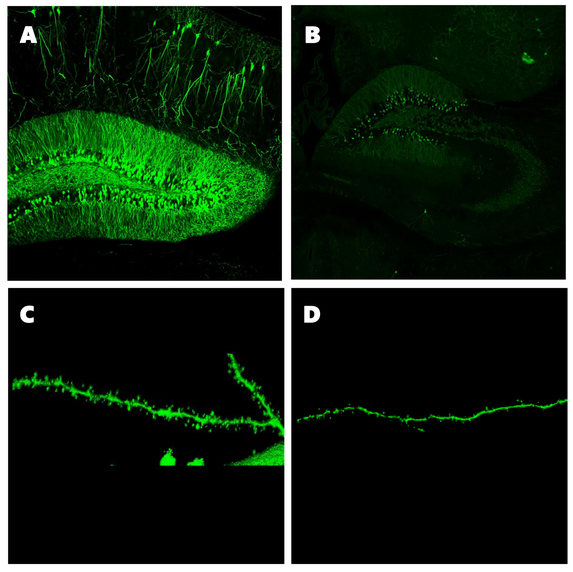

Figure 1. Comparison of Tissue Clearing Images to a Conventional Staining Technique of neurons in 5xFAD mouse hippocampus. Hippocampal neurons from the conventional staining technique are shown in green in panel A , and panel B shows the same hippocampal section but from the tissue clearing technique. Panel C shows a dendrite with dendritic spines from the conventional staining method, with panel D showing the same dendrite from the tissue clearing technique.

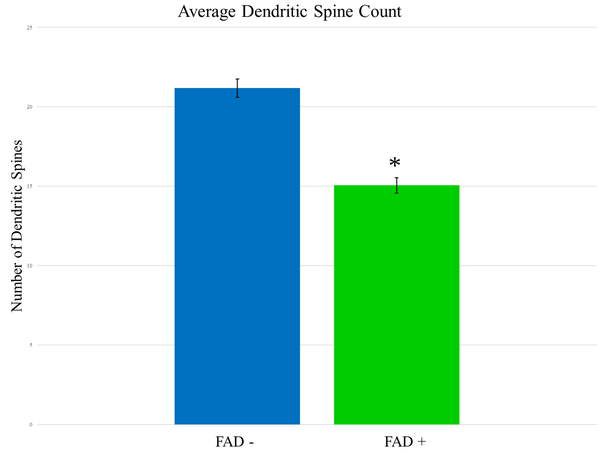

Figure 2. The Effect of Amyloid Beta on Dendritic Spine Number. FAD+ mice exhibited a significant decrease in dendritic spine number compared to the FAD- control. * represents a statistically significant difference at $p < .005$. Bars represent mean ± SEM.

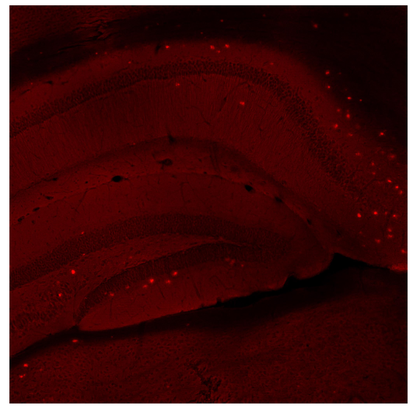

Figure 3. Amyloid Beta in a FAD+ Mouse Hippocampus. Red indicates the presence of amyloid beta. Bright red spots signify amyloid beta plaques.

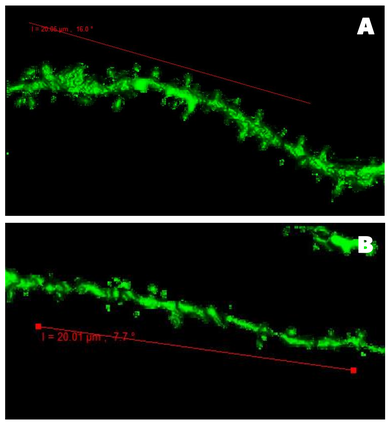

Figure 4. Comparison of Neurons in FAD- and FAD+ Mice. Panel A shows a neuron of a FAD- mouse. Panel B shows a neuron of a FAD+ mouse.

Conclusion

Our results from the clearing technique showed that this procedure did not provide an advantage in imaging resolution, in fact, the conventional staining provided better results. The MeOH treatment administered diminished the intensity of the fluorescent protein and caused the GFP antibodies to give off a poor signal.
We hypothesized that increased levels of Aβ levels in the FAD+ mice would cause a decrease in the number of dendritic spines. Our hypothesis was supported. A statistically significant decrease in the number of dendritic spines per neuron was observed in the FAD+ mice compared to the control group. Amyloid beta, was observed throughout the hippocampal region in the FAD+ mice. No Aβ was observed in the brains of the FAD- mice. The presence of Aβ coupled with the decrease in dendritic spine number indicates that Aβ likely has a role in causing a decrease in synaptic density.

Future Directions

The adaption to our 50 micron brain sections was not achieved but we may be able to utilize the clearing tissue technique with whole brain samples and with a 2-photon microscope, which is able to penetrate deep into tissue.
Our lab has previously used an LPS model to induce amyloid beta production in WT mice. Future studies could examine neurons in mice treated with LPS to see if the amyloid beta production and observed cognitive deficits correlate with a decrease in synaptic density.

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