The Survival of Healthy (Tau-) Neurons in Tau-stained CA1 Region of Nondemented Individuals (NDAN) with Alzheimer’s Histopathology

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Abstract:
Non-Demented Alzheimer’s Disease (NDAN) individuals are characterized by having Alzheimer’s Disease histopathology of amyloid plaques and neurofibrillary protein tangles made up of Tau proteins but not suffering from dementia. We analyzed brain tissue samples in an area of the hippocampal subfield called CA1 which is an area that is sensitive to cognitive decline and demonstrates significant neurodegeneration caused by Alzheimer’s Disease. We counted and measured healthy neurons in the CA1. We correlated the number of healthy neurons in these sensitive areas with people diagnose with Alzheimer’s Disease but were not demented (NDAN). Tau was specifically analyzed to confirm that the tissue samples that were analyzed, originated from individuals that suffered from AD histopathology. Comparing the Tau-stained hippocampal tissues from AD, NDAN, and control cases we observed that the number of Tau negative (Tau-) healthy neurons were present in AD cases, but significantly higher in NDAN, and in controls. In contrast, Tau positive (Tau+) presumptive unhealthy neurons were most prevalent in AD and NDAN cases, but far less numerous in controls. The decreased number of healthy neurons found in AD cases and increased healthy neuron presence in NDAN cases might provide an explanation as to why NDAN individuals did not suffer from dementia. These observations are consistent with HIF2A results described by Vivianne Mitri in her recent (2020) thesis “Overexpression of HIF-2a in Protected Regions of Alzheimer’s Disease in Resilient Cases”.

Introduction:
Alzheimer’s Disease based on NIA-Reagan histopathology criteria is defined by the amount of and extent of distribution of amyloid plaques and neurofibrillary tangles (NFTs) in the brain at autopsy. However, the clinical diagnosis of Alzheimer’s disease along with includes the
expression of cognitive decline or dementia. Since about 60 to 70% of individuals who have dementia are also diagnosed with having AD histopathology, Alzheimer’s disease is often described as the leading cause of dementia. Until recently, most people assumed that if a person had Alzheimer’s Disease, they also suffered from dementia. However, recent studies have demonstrated that there are many individuals who had led normal productive lives (such as some “superagers” or individuals in the 90+ study) and were cognitively intact at the time of their death, expressed all of the hallmarks of Alzheimer’s disease histopathology upon autopsy.

Individuals that have the histopathology of Alzheimer’s Disease but did not suffer from dementia are referred to as Non-demented individuals with Alzheimer’s Disease or NDAN. Individuals with Alzheimer’s histopathology and dementia are referred to as AD individuals.

The reason CA1 was selected for the present study is because of its selective vulnerability to neurodegenerative conditions such as hypoxia, vascular disease and Alzheimer’s disease. One of the reasons for CA1’s selective vulnerability is likely due to its relatively low oxidative metabolic capacity as determined by cytochrome oxidase histochemistry (Kageyama and Wong-Riley, 1982). There is now evidence that CA1 pyramidal cells have the ability to overcome their relatively low oxidative capacity by undergoing a metabolic reprogramming associated with enzymatic changes involved with hypoxic adaptation (Mitri, 2020 - Kageyama, advisor).

**Tau**

Tau is a microtubule associated protein (sometimes referred to as MAP tau) that polymerizes to form neurofibrillary tangles (NFTs, Ittner and Gotz, 2011, Scheltens et al., 2016). One of the areas of the brain that appears to be the hardest hit by Alzheimer’s histopathology, is the CA1 region of the hippocampal formation. There have been many descriptions of amyloid plaques and
Analyzing Tau in CA1

Tau-based NFTs and its involvement with CA1 in Alzheimer’s Disease, but there has not been an analysis which includes cell measurements and cell counts of Tau negative healthy neurons in Tau-stained tissue preparations. Most reports have claimed that many neurons in CA1 had shrunk or degenerated in AD, but few have reported the numbers of healthy neurons that remained. In order to determine if the number of healthy neurons in cognitive processing areas of the brain could be related to cognitive health of the individual, the present study involved an analysis of the relative numbers and sizes of both AD-afflicted Tau+ neurons, and presumptive normal healthy Tau-negative (Tau-) neurons in the CA1 region of the hippocampal formation in AD, NDAN and normal control brains. The present study not only confirmed previous AD findings in literature but also provided evidence that amidst the presence of abundant Tau histopathology in CA1, a significantly greater number of presumptive healthy Tau-negative neurons were observed in the nondemented (NDAN) cases compared with demented AD cases in the CA1 region of the hippocampus.

Methods:

Formalin-fixed paraffin-embedded (FFPE) 6 µm slide mounted tissue from postmortem human subjects were obtained by Banner Sun Health which was then sent by NDB Bio for immunohistochemical processing. The AD, NDAN, and control tissues were stained with an antibody to phospho-Tau, which is used as a marker for AD histopathology. In Vivianne Mitri’s work she mentioned that HIF-2a in the nuclei was stained CA1 neurons which are known to be protected in NDAN. With the training of Dr. Kageyama and Ayah Elsamad the researchers took on the analysis of the immunohistochemical data, by identifying and counting Tau positive (Tau+) and presumed normal unstained Tau negative (Tau -) neurons in the tissue samples. Then cross-sectional neuron diameter measurements were obtained. In order to maintain the integrity
of the results this was conducted as a double-blind study. The scanned digital images for this research were exclusively from the CA1 region of the hippocampal formation. Students were trained to distinguish neurons from other cell types in the CA1 region of the hippocampus. Other cell types included glial cells, such as astrocytes, oligodendrocytes and microglial cells, and vascular cells, such as the endothelial cell, pericyte, smooth muscle cells and fibroblasts. The Tau+ neurons were subclassified as “D” for darkly stained, “M” for moderately stained, “L” for lightly stained, and “Tau-” for normal unstained healthy neurons, and were also counted. Next, the ten most prominent healthy Tau negative neurons were measured from each image in millimeters (to the nearest 0.1 mm), and then converted into microns. Ayah Elsamad then collected the data from the researchers and was made aware by Dr. Kageyama whether the tissue sample examined pertained to AD, NDAN, or control and then created a quantitative analysis from the data collected from the CA1 Tau counts, categorized them appropriately and obtained the average and standard deviation of each slide and then each case with both the cell measurement and cell count. Our research derived our information from nine cases overall, three of them were AD, two of them were NDAN and four cases pertained to our control group.

**Results:** Cell counts per standardized sample area (200,000 µm²)

When comparing neurons in AD, NDAN, and control, the mean number of presumptive healthy (Tau -) neurons were the lowest in AD cases, higher in NDAN and highest in control per standardized sample area of 200,000 µm². When looking at Tau - in AD we got a value of 4.67 ± 5.59, for Tau+ in AD we got a value of 19.65 ± 4.98 in total for AD the value was 24.32 ± 7.89. For NDAN Tau- we got a value of 15.57 ± 7.55, for Tau+ it was 11.32 ± 1.92 and the total value was 26.90 ± 8.95. For control the values of Tau- counts was 24.97 ± 10.28, for Tau+ it was 6.79 ± 4.14, the total value was 31.77 ± 9.24.
Figure 1: CA1 Tau Counts

Differences in Cell Size

The measurements of the ten most prominent healthy (Tau-) neurons taken demonstrated that the largest neurons were contained in the NDAN individuals, the second largest overall was presented by the AD individuals and lastly the control individuals had the smallest (Tau-) measurements overall. For the cell measurements the mean size for neurons measured in AD was \(14.72 \pm 2.75\ \mu m\). NDAN neurons had a mean size of \(17.03 \pm 1.79\ \mu m\). The control group mean neurons size was \(13.36 \pm 1.35\ \mu m\).


**Discussion:**

We observed that the AD cases had the highest number of Tau+ neurons compared to NDAN and controls. AD cases also had the lowest mean numbers of healthy Tau- neurons and total neurons per sampling area compared with NDAN and control cases. It is assumed that the high number of Tau+ neurons in AD cases resulted in the significant neuronal loss in CA1 which eventually led to a significant shrinkage of the entire CA1 laminae. The most important observation was the presence of a large number of presumed healthy Tau- neurons present alongside numerous AD afflicted Tau+ neurons in the CA1 region of the NDAN cases. Since the Even though the NDAN cases expressed a high level of AD histopathology, the presence of large numbers of healthy Tau- neurons could be the reason that NDAN individuals did not suffer from dementia.

When we measured the mean cross-sectional diameters of the surviving healthy Tau- neurons the mean size of the Tau- CA1 neurons in AD and NDAN cases was larger than the Tau- CA1 neurons in the normal control cases.

We hypothesize that Tau- CA1 neurons in AD and NDAN cases were larger than the neurons in control because the neurons that were able to survive the neurotoxic effects of Tau, were also able to not only maintain their original size, but were able to grow in size (undergo hypertrophy) in response to extending their axons into neighboring areas vacated by degenerating Tau+ neurons. Reactive synaptic rearrangement and synaptogenesis is a common phenomenon that has been described in a number of brain areas. A measurement of all the healthy (Tau-) neurons contained in each slide might have provided a better representation of all neurons, compared to the measurement we conducted of the ten largest ones, but the addition of measuring large numbers of potential Tau- degenerating/shrinking neurons would have diluted the effect for the
detection of neuronal hypertrophy. The hypertrophy of the healthy neurons in NDAN (and AD) may have taken over the functions vacated by the degenerating neurons; they might have also taken up synaptic space and formed more connections and because of the added connections might have grown larger.

**Conclusion:**

It is widely believed that AD histopathology is accompanied by a loss of functional neurotransmission and hypometabolism with resulting development of cognitive loss or dementia. Along with the previous statement, it is believed that the neurovascular damage caused by AD related proteins Aβ and MAP tau are made possible by a variety of AD related genes such as PSEN1, PSEN2, APOE4, APP, ultimately resulting in neuronal loss due to damaged blood vessels, reduced blood flow and/or hypoxia. Our work is consistent to observations by Vivianne Mitri (2020) who demonstrated that healthy neurons in non-demented AD (NDAN) cases expressed elevated levels of hypoxia inducing factor 2a (HIF-2a) in CA1 and the subiculum. Our work is also consistent with several reports of the benefits of hypoxia adaptation to surviving neurons and the prevention of reduction of cognitive deficits followed by the neuroprotective effects of prior exposure to ministrokes. The present study demonstrates that many neurons can survive and even thrive against the neurodegenerative effects of AD histopathology. The present study also reveals the possibility that we might be able to do something to prevent dementia, even if we are unable to stop AD histopathology. We propose that mental exercise naturally induces “functional” hypoxia which could then stimulate alternative metabolic pathways which these neurons can readily use to protect themselves from the neurotoxic effects of Tau or Amyloid plaques, so by this logic engaging in continual mental activities can increase the probability of neuron survival.
While most past research funding has been devoted to AD research by eliminating or suppressing the effects of Aβ or amyloid plaque formation, or the progression of tau pathology, very little funding has been devoted to preventing dementia by the promotion of neuronal survival. This needs to change.

References:


Mitri, Vivianne (2020) Overexpression of HIF-2a in Protected Regions of Alzheimer’s Disease Cases, CPP Master’s Thesis, Advisor, Dr. Kageyama)