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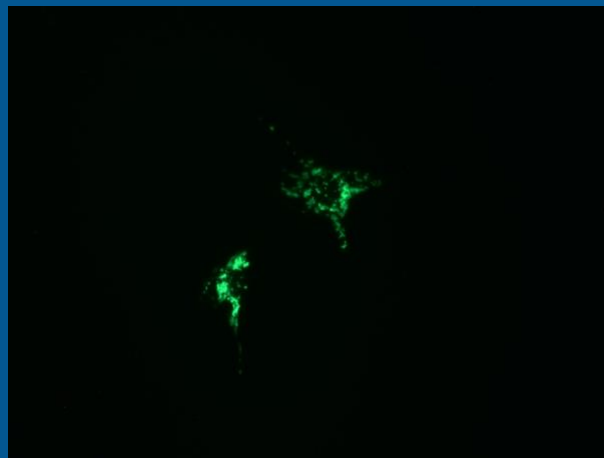

Preliminary study of the effect of tau-441 on mitophagy in *Gallus gallus* sympathetic neurons

Arianna J. Pesarik

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that is typically associated with severe memory loss, spatial disorientation, and an overall gradual deterioration of thought capacity. (Medeiros, Baglietto-Vargas, & LaFerla, 2011). Alzheimer's is often identified by two common characteristics: ß-amyloid plaques and intracellular neurofibrillary tangles, or NFTs (Gómez-Ramos, Díaz-Hernández, Cuadros, Hernández, & Avila, 2006). These tangles are composed of hyper-phosphorylated tau proteins, which are also a defining feature of other neurodegenerative diseases, such as Chronic Traumatic Encephalopathy, also known as CTE (Albayram et al., 2016).

One very important difference between the plaques and tangles is the difference in distribution. The beta-amyloid plaque distribution in the brain can be quite unpredictable, both in each affected individual, and in the exact brain regions where the plaques are found (Gómez-Ramos et al., 2006). NFTs, however, are typically found to form in a pattern, beginning in the entorhinal cortex, and then moving to the hippocampus and other surrounding areas (Gómez-Ramos et al., 2006). The formation of NFTs has a distinct correlation with the progress of Alzheimer's, since in regions of the brain such as the hippocampus there is an inverse relationship between the amount of extracellular tangles and the number of surviving cells. (Gómez-Ramos et al., 2006). Tau is a microtubule-associated protein that forms insoluble filaments, which eventually accumulate into neurofibrillary tangles in Alzheimer's disease (Medeiros et al., 2011). Tau maintains the structural stability and assembly of microtubules within the cell. In a brain affected by AD (and other neurodegenerative diseases), however, tau molecules aggregate into helical filaments, causing the microtubules within the cell to disassemble (Medeiros et al., 2011).

Mitophagy is the process within the cell to regulate cellular energy, homeostasis, and cell death (Ding & Yin, 2012). The removal of damaged mitochondria through autophagy, a process called mitophagy, therefore is essential in order to maintain a cell's proper functioning (Ding & Yin, 2012). I will be using mitophagy to determine cellular stability.

In this study, we treated Gallus gallus sympathetic neurons with a Tau-441 solution and a Rhodamine-123 solution, respectively. We will use Rhodamine-123 to stain the mitochondria in order to measure the fluorescence (Ding & Yin, 2012). In this study, I tested the hypothesis that the addition of the Tau-441 protein solution, leading to an increase in intracellular tau, would result in a decrease in cellular mitophagy. Tau protein aggregation has been studied, and found to be linked to Alzheimer's progression (Medeiros et al., 2011). Therefore, if we can determine the

amount of tau protein found within the cells, we can track, and perhaps even prevent, Alzheimer's progression.

Materials and Methods

For this experiment, we tested the effects of a 33 µl concentration of Tau-441 protein solution on cellular mitophagy in sparse dishes of 2-day old and 9-day old neuronal cells.

Primary Culture

Primary cell tissue culture was performed on ten-day old *Gallus gallus* neurons and glial cells, a technique described and developed by Morris (2015a). Coverslips containing sparse groups of neurons and glial cells were used for imaging and analysis.

Preparation of Tau-441 solution

The Tau-441 stock solution was made by reconstituting one tube of 50µg Sigma Aldrich Tau-441 powder in 50 µl sterile water, creating a 1mg/ml stock solution . Once the stock solution was made, 33µl of it was added to 1 ml growth medium. This solution was applied to the cells using a full exchange method, switching the growth medium the cells were already in with the tau-treated growth medium solution. Tau-441 solution

Preparation of Rhodamine-123 solution

To create the Rhodamine working solution from the Rhodamine-123 1mg/ml stock solution, we added 1µl pre-made stock solution to 1 ml growth medium for every dish that would be treated with the solution. In the case of our experiment, this meant adding 4µl of stock solution to 4 ml growth medium. The solution for Rhodamine-123 used in this experiment was developed by Daniel Southerland, Matthew Morgan, Omar Raouf, Luis Lazo, and Stephanie Martin.

Cell Staining

For the cell staining using the Rhodamine-123 working solution, we removed the growth medium from the sparse petri dishes containing the tau-treated cells and replaced it with the 1ml Rhodamine-123 growth medium solution, a full exchange. We then covered the dishes with aluminum foil, and let them incubate for 10 minutes at 37 degrees Celsius. After the incubation period, we washed 4 times with Hank's Best Salt Solution, and prepared a chip chamber with the cells.

Cell observation using chip chambers

Cells were plated and observed, using the chip chamber method, as described by Morris (2015b).

Fluorescent Microscopy and Imaging

Cellular imaging and observation was done in the Imaging Center for Undergraduate Collaboration (ICUC) at Wheaton College, using an upright Nikon Eclipse E200 Microscope using the 40x lens and a Nikon microscope 1.0x C-mount. A Spot Insight FireWire 2 camera was connected to the C-mount. The images were taken using Spot software, version 5.2, on an Apple iMac Macintosh computer, OS X Yosemite, version 10.10.5.

Image Analysis
Image analysis was done for each sample by calculating the fluorescence of each image using Fiji, also known as ImageJ, software. Imaging was done using method mentioned by Camilla Christina Pedersen in the paper (Pedersen, 2017).

Results

The amount of vesicles counted within the cells seemed to be lower in the tau-treated cells than in the cells not treated with tau. This could possibly mean that the tau solution actually decreased the level of mitophagy within the cell.

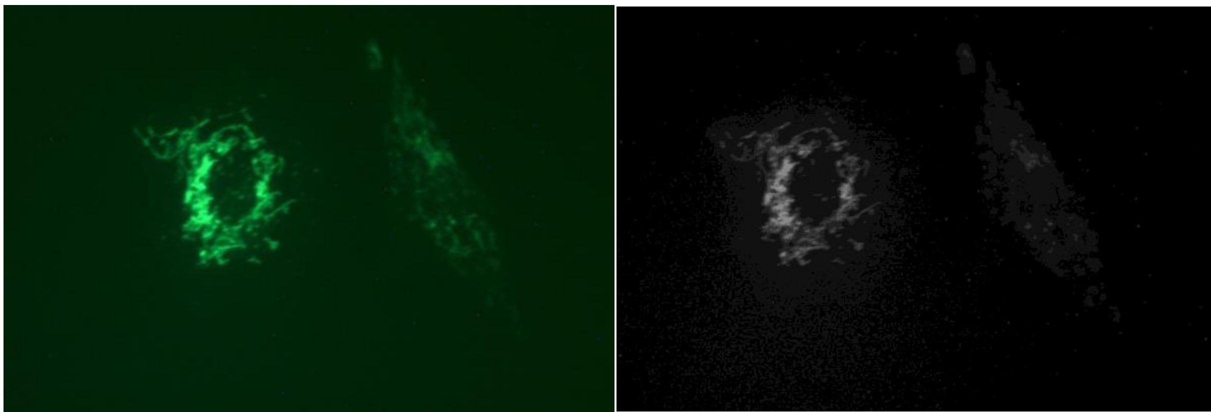

Figure 1. Control: A 2-day old neuron treated with just Rhodamine-123 solution, and no Tau-441. Photos taken before using ImageJ and after, respectively. The white portion of the right image shows the brightness measured by the software. Compared with other taken images of difference cells, the brightness of this cell is relatively low. This image was taken in collaboration with Matthew Morgan and Daniel Southerland.

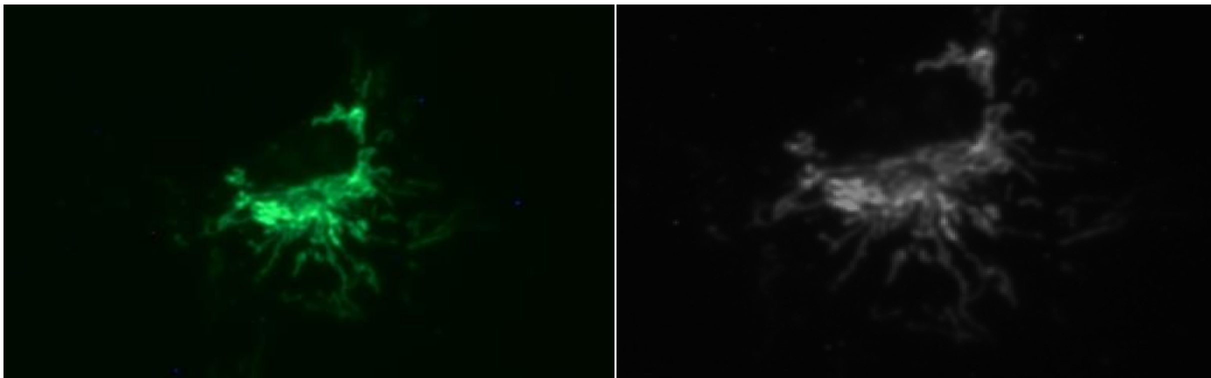

Figure 2. A 2-day old neuronal cell treated with Tau-441 solution, and then stained with Rhodamine-123 solution. We can see that this image is significantly brighter than the image taken of the control. This image was taken in collaboration with Matthew Morgan and Daniel Southerland.

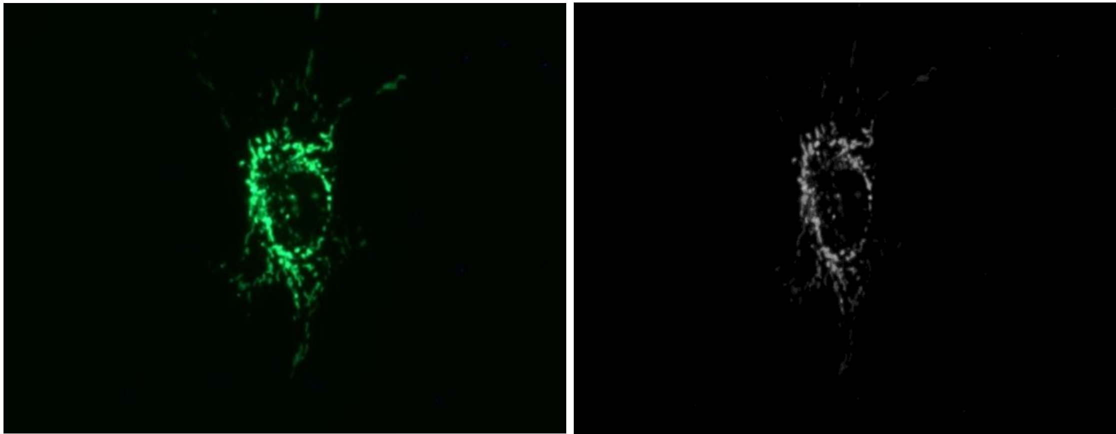

Figure 3. 9-day old neuronal cells treated with the Tau-441 solution. These cells were also treated with Rhodamine-123 for staining. These images were measured as brighter than the control, but less bright than the 2-day old cells. This image was taken in collaboration with Matthew Morgan and Daniel Southerland.

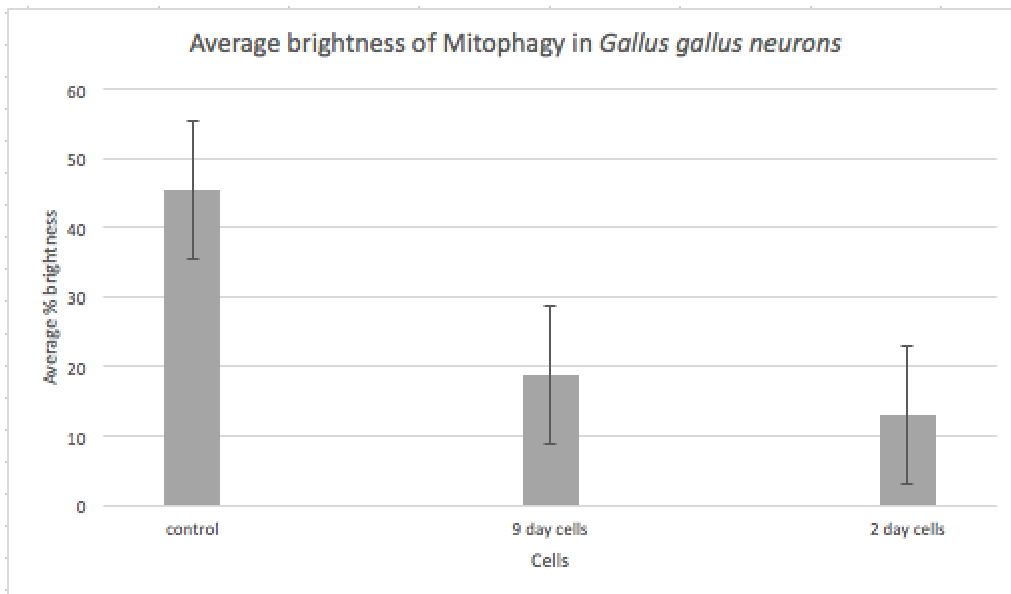

Figure 4. The average counted number of vesicles within each type of treatment. Bar 1 is the Control, untreated by tau, and only treated by Rhodamine 123. Bar 2 is 2-day old cells, treated with both Tau and Rhodamine-123 solutions. Bar 3 is 9-day old cells, treated with both Tau and Rhodamine-123 solutions. The graph includes error bars for each average. Fluorescence was brightest when measured using Fiji software, in the control cells. Fluorescence in mitochondria was also brighter in 9-day cells than 2-day cells.

Discussion

Through observation of the cells, the tau-treated cells appeared brighter on the imaging software than the non-tau-treated cells.
In conclusion, using this sample size of data, the control was significantly dimmer, while the cells treated with tau were significantly brighter. This could possibly be evidence that Tau-441 increases mitophagy, but it is difficult to know for sure with a small amount of data. To get more conclusive results, more data is needed. A significantly larger sample size is needed to make any firm conclusion about the results within this experiment. Using a larger sample size, we could also perhaps discover a difference between mitophagy in 2-day and 9-day old tau-treated cells. Using this data, it appears as if there was more mitophagic activity in the 2-day old cells than the 9-day old cells. However, a definite argument cannot be made concerning this without further data and evidence supporting this conclusion.

In the future, I would recommend testing several concentrations of tau at once, in order to determine at what level of tau does the amount of vesicles within the cell begin to decrease. If we can determine at what level/concentration that tau starts to have a negative effect on the cell, we can determine in the future, at what point we must intervene to prevent damage to the cells.

Using this data, we could possibly find a new way to treat Alzheimer's. If we somehow find the technology to determine levels of tau protein in the brain, sometime in the future, we may be able to determine how long a person has before their Alzheimer's starts to become severe.

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All experimental procedures, including cell treatments with Tau-441 and Rh-123, were done in collaboration with Matthew Morgan and Daniel Southerland. Cellular imaging technique was developed by Matthew Morgan and Daniel Southerland. The solution for Rhodamine-123 used in this experiment was developed by Daniel Southerland, Matthew Morgan, Omar Raouf, Luis Lazo, and Stephanie Martin.

I have abided by the Wheaton College Honor Code
Arianna J. Pesarik