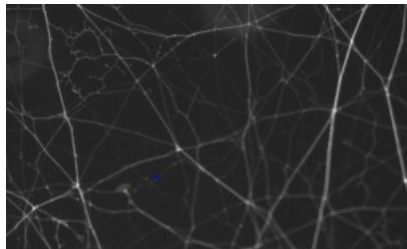

Analyze Nerve Growth Factor (NGF) deprivation in axonal degeneration

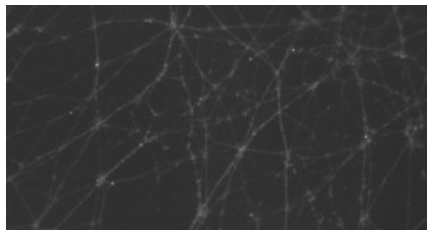
This is for the course BIO:4386 Intro to Scientific Computing in the Spring of 2023. This aims at recreating the graph from the unpublished data behind the NGF Deprivation Experiment (Dr. Daniel Summers Lab).

Introduction

Alzheimer's disease is one of the most common forms of dementia. It is onset by multiple factors but most commonly seen in loss of neuronal connectivity due to axonal degeneration. While there are many factors of neurodegeneration, our lab studies the mechanism for loss of axonal connectivity termed Wallerian degeneration. Wallerian degeneration refers to physical damage inflicted on an axon that induces degeneration. This physical damage causes anterograde degeneration, i.e., degeneration towards the distal axon terminal. This is distinguished from retrograde degeneration, which is when degeneration occurs towards the proximal cell body. The effects of the two degeneration are different only in terms of where the degeneration starts and the direction it goes in. Despite physical damage in axons being uncommon, Wallerian degeneration is still very similar to how axons react when undergoing a traumatic injury in the central nervous system and an efficient model for our lab.



An image collected from ImageJ of healthy axons, notice the thicker, brighter, stronger axons in the image.



An image collected through ImageJ of unhealthy axons showing signs of degeneration through thinner lines, degraded areas, and the axons beginning to shrivel as they fall apart.

Anterograde degeneration is seen in different peripheral neuropathies and diseases

and is the cause for diseases such as Alzheimers and/or ALS. For this experiment, our goal is to look at how anterograde degeneration induced by Wallerian degeneration is affected by either having, or not having supplemental Nerve Growth Factor (NGF) added. NGF is a protein that promotes cell growth. NGF has many important regulatory functions in nerve cells in the peripheral and central nervous system. It is normally added to cells while being cultured to help them survive. To induce the Wallerian Degeneration, the axons are cut just slightly above the soma. This cut is the traumatic injury that the cells will undergo. For this study, we are looking to see if NGF has an influence on neuron cell death or survival during Wallerian Degeneration. Since NGF is a neuron survival factor that we normally add while culturing the cells to promote cell growth due to its regulatory functions, we want to study the effect NGF can have on axons. Our hypothesis for this experiment was to see if there is an increase of growth in axons that have started to degenerate from physical damage when they have been deprived of NGF and had NGF added back to it later. NGF is not normally produced after injury, so this is to test for a possible repair mechanism.

From there we can take images of the cells and run the images through a protocol that can quantify the amount of degeneration that occurs in the cells using ImageJ. To do this, there is a protocol our lab uses that analyzes the amount of area from the image, and calculates how much of the cell is taking up the area. As the axons die and degenerate, they break down and take up less area in the visual field of the image. The protocol quantifies a number for this, and while not perfect it does provide a quantifiable way of assessing the data rather than a qualitative method. Degeneration indexes under .3 are seen as not undergoing severe degeneration and images quantified at a degeneration index of over .3 are seen as undergoing severe degeneration. In Wallerian Degeneration the production of NGF is upregulated because NGF is necessary for the survival of these neurons. Because NGF is necessary for neuron survival we would expect that with NGF deprivation to see more apoptosis in the neurons of these conditions. We would expect to see a spike in the amount of degeneration in the NGF deprived condition in comparison to the not deprived NGF condition because without the deprivation, the cells are still receiving growth support from the NGF.

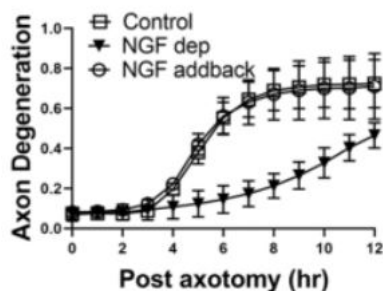


Fig. 3 This figure is unpublished and therefore does not have a figure legend yet. This figure shows the amount of Axon Degeneration scored on the degeneration index between 0.0-1.0 with scores above 0.3 to be considered undergoing induced/severe degeneration rather than normal cell decay. Along the X- axis is the hour post axotomy of the cells. Three variables are present, the control being the non NGF deprived cell cultures, NGF deprived cultures and NGF that was deprived and later added back to the cells.

This figure really addresses the question behind what can NGF do, as well as what influences axonal degeneration. While it would have been expected that cultured cells deprived of NGF would have higher axonal degeneration (above .3 in the degeneration index) because NGF, a cell growth promoting protein, normally supports cell growth. It was found that the NGF deprivation would in fact lower the amount of axonal degeneration, and slow down the rate of degeneration compared to other groups such as the control group and the NGF addback group. What's the current hypothesis with respect to this observation? <— need to speak to Dr. Summers

Materials and Methods

Data

Data was given in a .csv file from Dr. Summers. Data for only the NGF addback group is uploaded to github repo with an annotated file explaining how the data will be wrangled to proceed in making the figure.

1. Access the github repo through the link: <https://github.com/Intro-Sci-Comp-UIowa/biol-4386-course-project-maddemir>
2. Click on the data folder
3. Click on the DWS 121 NGF addback Bclxl timecourse.csv file
4. You'll see the file pull up, and we will focus on the columns listed as filename as well as degeneration index.

Methods:

My end goal with this project is to find an efficient way of organizing data and using data wrangling techniques so that it can be reproducible/usable by others in my lab. I would like to also incorporate calculating the averages of the degeneration index for each well and each hour in the timecourse using all eighth visual fields in each well.

Filename:

A10_03_1_1_Texas Red_001.tif

Well Visual field number Hour #

Well A10

- Visual field 1
 - Images for hours 1-13
- Visual field 2
 - Images for hours 1-13
 - Images for hours 1-13
- Visual field 4
 - Images for hours 1-13
- Visual field 5
 - Images for hours 1-13
- Visual field 6
 - Images for hours 1-13
- Visual field 7
 - Images for hours 1-13
- Visual field 8
 - Images for hours 1-13
- Average deg index for hour 1 from all 8 visual fields
- Repeat for all 13 hours

To achieve this, I will be taking the raw data from a figure with three variables and focusing on one variable; NGF addback. My goal is to write a code through R/R studio to take the data after it has been quantified into a .csv file. Example using columns from .csv file visual fields 1-2 for well A10 (there are eight visual fields, only used two in this example)

Step 1: Through R, I will separate all wells, A10, A11, A12 etc .

Step 2: There are eighth visual fields for each well. In each visual field there is an image taken for hours 1-13

Step 3: I will take the average of all the images for each hour from all the visual fields.

Step 4: Creating a visual of the means

Step 1. Here all of the A10 wells are grouped together

filename	Area analy	TAA	FAA	IAA	degenerat	obj
A10_03_1_1_Texas Red_001.tif	3968064	180371	112300	68071	0.6226	0
A10_03_1_1_Texas Red_002.tif	3968064	224668	133296	91372	0.5933	0
A10_03_1_1_Texas Red_003.tif	3968064	214835	128022	86813	0.5959	0
A10_03_1_1_Texas Red_004.tif	3968064	213901	127849	86052	0.5977	0
A10_03_1_1_Texas Red_005.tif	3968064	214175	125819	88356	0.5875	0
A10_03_1_1_Texas Red_006.tif	3968064	236849	141621	95228	0.5979	0
A10_03_1_1_Texas Red_007.tif	3968064	225580	133973	91607	0.5939	0
A10_03_1_1_Texas Red_008.tif	3968064	210315	125303	85012	0.5958	0
A10_03_1_1_Texas Red_009.tif	3968064	191617	114060	77557	0.5952	0
A10_03_1_1_Texas Red_010.tif	3968064	230942	137412	93530	0.595	0
A10_03_1_1_Texas Red_011.tif	3968064	183442	107173	76269	0.5842	0
A10_03_1_1_Texas Red_012.tif	3968064	243217	140053	103164	0.5758	0
A10_03_1_1_Texas Red_013.tif	3968064	215767	129218	86549	0.5989	0
A10_03_1_2_Texas Red_001.tif	3968064	238928	145897	93031	0.6106	0
A10_03_1_2_Texas Red_002.tif	3968064	198664	122063	76601	0.6144	0
A10_03_1_2_Texas Red_003.tif	3968064	212708	130835	81873	0.6151	0
A10_03_1_2_Texas Red_004.tif	3968064	220457	133354	87103	0.6049	0
A10_03_1_2_Texas Red_005.tif	3968064	229105	140166	88939	0.6118	0
A10_03_1_2_Texas Red_006.tif	3968064	268984	161709	107275	0.6012	0
A10_03_1_2_Texas Red_007.tif	3968064	220953	133850	87103	0.6058	0
A10_03_1_2_Texas Red_008.tif	3968064	227005	135319	91686	0.5961	0
A10_03_1_2_Texas Red_009.tif	3968064	247212	148579	98633	0.601	0
A10_03_1_2_Texas Red_010.tif	3968064	244063	147951	96112	0.6062	0
A10_03_1_2_Texas Red_011.tif	3968064	220319	131330	88989	0.5961	0
A10_03_1_2_Texas Red_012.tif	3968064	238082	142642	95440	0.5991	0
A10_03_1_2_Texas Red_013.tif	3968064	266067	156772	109295	0.5892	0

Step 2 . Visual fields have been separated

Visual field 1:

filename	Area analy	TAA	FAA	IAA	degenerat	obj
A10_03_1_1_Texas Red_001.tif	3968064	180371	112300	68071	0.6226	0
A10_03_1_1_Texas Red_002.tif	3968064	224668	133296	91372	0.5933	0
A10_03_1_1_Texas Red_003.tif	3968064	214835	128022	86813	0.5959	0
A10_03_1_1_Texas Red_004.tif	3968064	213901	127849	86052	0.5977	0
A10_03_1_1_Texas Red_005.tif	3968064	214175	125819	88356	0.5875	0
A10_03_1_1_Texas Red_006.tif	3968064	236849	141621	95228	0.5979	0
A10_03_1_1_Texas Red_007.tif	3968064	225580	133973	91607	0.5939	0
A10_03_1_1_Texas Red_008.tif	3968064	210315	125303	85012	0.5958	0
A10_03_1_1_Texas Red_009.tif	3968064	191617	114060	77557	0.5952	0
A10_03_1_1_Texas Red_010.tif	3968064	230942	137412	93530	0.595	0
A10_03_1_1_Texas Red_011.tif	3968064	183442	107173	76269	0.5842	0
A10_03_1_1_Texas Red_012.tif	3968064	243217	140053	103164	0.5758	0
A10_03_1_1_Texas Red_013.tif	3968064	215767	129218	86549	0.5989	0

Visual field 2:

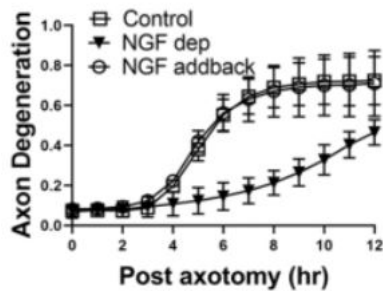
A10_03_1_2_Texas Red_001.tif	3968064	238928	145897	93031	0.6106	0
A10_03_1_2_Texas Red_002.tif	3968064	198664	122063	76601	0.6144	0
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A10_03_1_2_Texas Red_013.tif	3968064	266067	156772	109295	0.5892	0

Avg hour 1 in well A10: $(.6226+.6106)/2 = .61660$

Avg hour 2 in well A10 : $(.5933+.6144)/2 = .60385$

Continue until hour 13

Step 4: Graphing mean values



Reflections:

- I am unsure if I did the data part correctly/ what was expected, mostly because I didn't download the data from online. I hope what I did was sufficient and is reproducible but am unsure if I needed to include more info about experiments. I am definitely not confident I am meeting expectations in that section.
- I am still figuring out how to reproduce the figure, I've been working with Yann as well as working on the coursera course to keep learning how to utilize R in order to learn everything I need to so I can write a code to wrangle the data the way I need to.