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Effect of Glaucoma on Melanopsin Retinal Ganglion Cells in Mice

Abigail McMullen
acm154@uakron.edu

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Honors Research Project

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Abstract

Within the mammalian retina, there are melanopsin-containing ganglion cells. These intrinsically photoreceptive cells help control behavioral aspects of vision as well as to aid in circadian rhythms. In this project, it will be determined whether or not glaucoma has an effect on these cells, and what that effect is, from different points in the disease. Glaucoma is an eye disease that damages the retinal ganglion cells and eventually causes them to die, also it can be linked to irregular circadian rhythm. Many techniques will be used to prepare this project but computer imaging will be the primary method for determining the effect glaucoma has on these cells. Computer imaging will be used to to trace neurons in control and experimental mice retinal ganglion cells. In addition to tracing, the method will be used to measure soma size of the neuron, the sum of its dendritic lengths, the dendritic field size, and the diameter of the dendritic field, in both groups in order to then run statistical data analysis and compare the results to see if glaucoma truly does have a significant effect on melanopsin retinal ganglion cells in mice.

Introduction

Glaucoma is an eye disease that damages the optic nerve and is present in 3 million Americans, acting as the second largest cause of vision loss and blindness (Vision Health Initiative, 2020) . For people that have glaucoma, about 50% of them are completely unaware -

this is because there are not typically any early symptoms so it is a very hard thing to catch (ibid). This disease damages the retinal ganglion cells and eventually causes them to die. Most cases of glaucoma are identified by an increase in intraocular pressure (IOP) as well as the axon layer of the retina changing (Shou et al., 2003). The elevation of IOP from glaucoma has been studied to show optic nerve fiber and retinal ganglion cell degeneration (Shou et al., 2003). The process of transporting materials from an axon to the cell body is called retrograde axonal transport. When the IOP levels are increased, this process is blocked which causes neurotrophin deprivation (Nickells, 1996). This hindrance of this transport leads to less neurotrophic factors in the retina, leading to death of retinal ganglion cells via apoptosis, and furthering the progression of the glaucoma disease (Claes et al., 2019).

Within the retina, there are many different kinds of retinal ganglion cells that could be damaged due to glaucoma. In this review, the focus is how glaucoma affects the photosensitive ganglion cells. These cells are important because they have melanopsin, their own photopigment used in a signaling cascade activation, which allows them to interact and respond to light without the presence of the rod and cone receptors as well as aid in the circadian rhythm cycle by opening up cation channels in the membrane (Sanes and Masland, 2015). In other words, these ganglion cells act as photoreceptors that are critically important in the cascade of phototransduction as well as the retinal photoentrainment pathway (Rollag et al., 2003). When these ganglion cells are damaged from glaucoma, their dendrites are shortened and do not function as they should, causing ongoing neurodegeneration which can lead to blindness (Agostinone and Polo, 2015). Many recent studies have been looked at to see the influence that these melanopsin ganglion cells have on behavioral functions such as contrast detection, color

discrimination, the enhancement of environmental scenes, and brightness perception (Sondereker et al. 2020).

It is important to use subjects that will correlate to human studies and future research. There are six different types of known melanopsin ganglion cells, M1-M6, in mice, and four in human retains, M1-M4 (Sondereker et al. 2020). It is shown in table 5 of the *Crosstalk* review, a summary of different species is shown for these known melanopsin ganglion cell types (ibid). Mice retinas and human retinas have similar morphologies and physiologies and therefore mice are good subjects to use for this study. More specifically, the change in retinal ganglion cell dendritic structure is important to study when looking at how glaucoma affects the process of visual information (Shou et al., 2003). In tact dendrites are used for cell to cell communication when they get the synaptic signals from retinal ganglion cells (Agostinone and Polo, 2015). Previous studies have concluded that Alzheimer patients typically have shorter dendrites with less branches and less spines (Einstein et al., 1994). In addition to dendritic length playing a role in Alzheimer patients, there have also been many studies that relate circadian rhythms dysregulation with Parkinson's and Alzheimer's disease - showing the loss of melanopsin ganglion cells (Sondereker et al. 2020). This is because even without functional photoreceptors, melanopsin ganglion cells are still able to communicate spatial information to the brain (Sondereker et al. 2020). Also, the response from melanopsin ganglion cells to light also has effects on cognition and alertness (Vandewalle et al., 2013).

This review adds to further research on the effects that glaucoma has on melanopsin-containing retinal ganglion cells. The focus is to conclude if the size of the neuron, the sum of its dendritic lengths, the dendritic field size, and the diameter of the dendritic field, are significantly different or not between normal and glaucoma induced cells. The null

hypothesis would be that there is no difference in the means between normal and glaucoma affected mice; the alternative hypothesis would be that there is a difference in the means between normal and glaucoma affected mice.

Methods

The mice used for all of these experiments - and kept in Akrons vivarium - were C57BL/6N mice, wild-type male and female (Sondereker, et al. 2017). Prior to this tracing experiment, the mice retina's were then extracted and prepared using dissection. There were control and experimental mice retinas. The experimental mice retinas were induced with glaucoma. The control mice retinas were ages 3 months, 5 months, and 7 months; the control mice retinas were ages 2 months, 4 months, and 6 months. There were also three different retinas (retina one, retina two, and retina three), each one sectioned into four quadrants (dorsal, ventral, nasal, temporal). This experiment used the control/normal mice retina from age 7 months, retina two, and the dorsal quadrant. The experimental/glaucoma induced mice retina that was used was age 6 months, retina two, and the dorsal quadrant. Twenty retina's were chosen from each group and traced using Fiji computer imaging. Data for soma size, total dendritic length, dendritic field size, and dendritic field diameter was collected using the software and then it was maintained in a mass google sheet spreadsheet. Graphpad was used to run normality tests for the data collected and StatCrunch was used to run non parametric tests (abnormal distribution) and two-sample t tests (normal distribution) for data analysis and interpretation.

Results

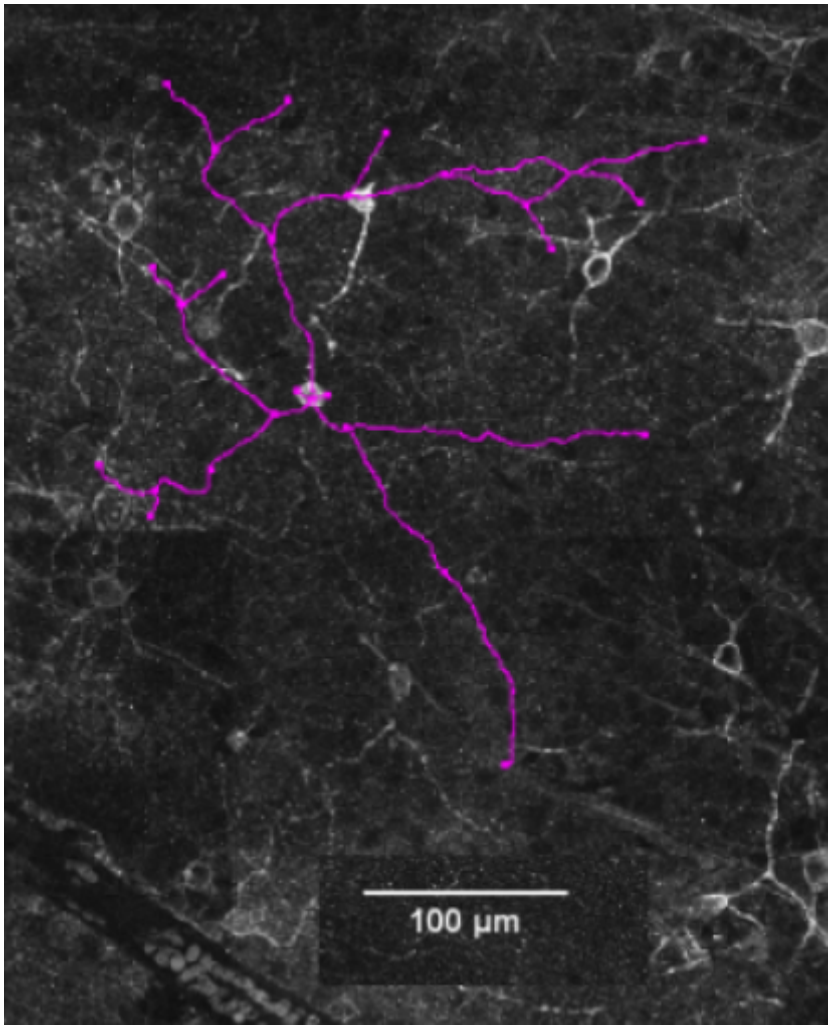

Figure 1: Tracing of a normal melanopsin retinal ganglion cell.

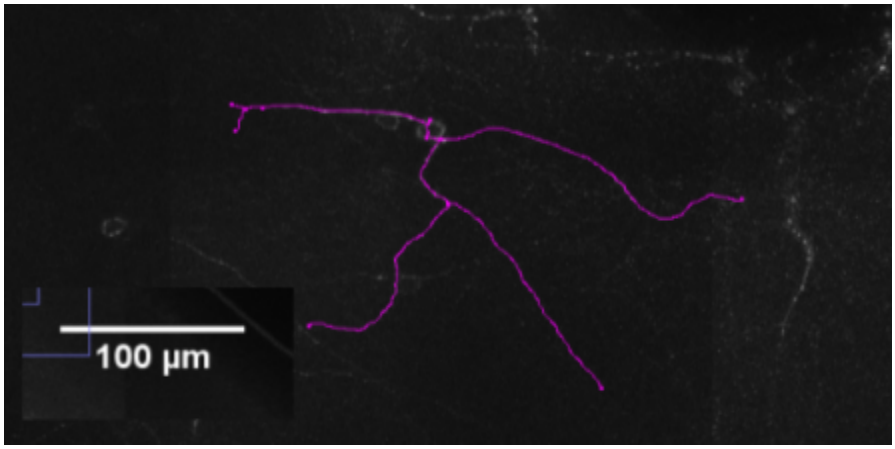

Figure 2: Tracing of a glaucoma affected melanopsin retinal ganglion cell.

Table 1: Raw data collected from Fiji computer imaging software. (All rounded to three decimal places)

Normal Soma Size	Glaucoma Soma Size	Normal Total Dendritic Length	Glaucoma Total Dendritic Length	Normal Dendritic Field Size	Glaucoma Dendritic Field Size	Normal Dendritic Field Diameter	Glaucoma Dendritic Field Diameter
12.581	14.645	732.070	653.069	20621.332	27395.630	162.037	186.765
13.489	11.815	1057.519	525.582	36567.118	6735.276	215.775	92.605
13.232	13.609	850.259	227.091	20836.108	2182.206	162.878	52.711
12.410	12.586	723.379	394.208	19413.215	9490.739	157.219	109.927
13.945	15.180	624.704	423.581	11501.561	10549.116	121.013	115.895
23.802	12.496	1520.630	289.260	46200.728	4775.026	242.538	77.973
18.406	13.254	971.858	290.609	32632.154	5616.29	203.835	84.563
13.400	14.797	945.680	414.863	24524.766	9901.561	176.709	112.281
16.129	14.828	772.621	439.899	19221.54	7495.734	156.440	97.693
15.820	10.951	820.006	473.999	19329.657	9285.016	156.880	108.729
12.060	13.733	816.473	490.816	27201.457	15034.651	186.102	138.357
11.959	14.709	792.682	702.790	23035.068	14092.092	171.258	133.950
13.824	12.318	652.392	255.318	27560.562	2576.691	187.326	57.278
15.816	10.950	447.621	383.978	8043.600	11021.748	101.120	118.462
14.417	8.752	627.980	839.910	13190.531	29910.718	129.594	195.150
12.639	8.560	959.774	370.510	30941.831	5827.784	198.485	86.140
12.055	10.670	616.687	500.499	14542.144	11472.841	136.072	120.862
13.535	8.538	1163.147	500.569	41841.519	10726.015	230.812	116.862
12.146	11.119	852.770	503.235	24264.100	12521.956	175.767	126.267
13.917	11.631	539.140	515.198	13079.292	16342.352	129.047	144.249

Table 2: GraphPad table showing the statistical breakdown of the tested groups to see if the results were normally distributed (passed the normality test or not).

Col. title	Normal Soma Size	Glaucoma Soma Size	Normal Total Dendritic Length	Glaucoma Total Dendritic Length	Normal Dendritic Field Size	Glaucoma Dendritic Field Size	Normal Dendritic Field Diameter	Glaucoma Dendritic Field Diameter
Mean	14.279	12.261	824.369	459.749	23727.414	11147.672	170.049	113.836
Standard deviation (SD)	2.793	2.131	241.39	150.45	10100	7120.3	36.905	36.056
Sample size (N)	20	20	20	20	20	20	20	20
Std. error of mean(SEM)	0.6246	0.4766	53.976	33.642	2258.3	1592.2	8.252	8.062
Lower 95% conf. limit	12.972	11.264	711.4	389.34	19001	7815.3	152.78	96.962
Upper 95% conf. limit	15.586	13.259	937.34	530.16	28454	14480	187.32	130.71
Minimum	11.959	8.538	447.62	227.09	8043.6	2182.2	101.2	52.711
Median (50th percentile)	13.512	12.407	804.58	456.95	21936	10225	167.07	114.09
Maximum	23.802	15.18	1520.6	839.91	46201	29911	242.54	195.15
Normality test KS	0.2476	0.1183	0.1532	0.1809	0.1185	0.1818	0.1062	0.1228
Normality test P value	0.0023	>0.10	>0.10	0.0855	>0.10	0.0821	>0.10	>0.10
Passed normality test?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 3: Non parametric hypothesis test result comparing normal soma size and glaucoma affected soma size.

Variable	n	n for test	Median Est.	Wilcoxon Stat.	P-value	Method
Normal Soma Size - Glaucoma Soma Size	20	20	1.7655	164	0.0266	Exact

Table 4: Two-sample t test hypothesis result comparing normal total dendritic length (μ_1) and glaucoma affected total dendritic length (μ_2).

Difference	Sample Diff.	Std. Err.	DF	T-Stat	P-value
$\mu_1 - \mu_2$	364.62024	63.602011	31.826123	5.7328414	<0.0001

Table 5: Two-sample t test hypothesis result comparing normal dendritic field size (μ_1) and glaucoma affected dendritic field size (μ_2).

Difference	Sample Diff.	Std. Err.	DF	T-Stat	P-value
$\mu_1 - \mu_2$	12579.742	2763.1552	34.145792	4.552673	<0.0001

Table 6: Two-sample t test hypothesis result comparing normal dendritic field diameter (μ_1) and glaucoma affected dendritic field diameter (μ_2).

Difference	Sample Diff.	Std. Err.	DF	T-Stat	P-value
$\mu_1 - \mu_2$	56.213338	11.536969	37.97941	4.8724528	<0.0001

The results on how glaucoma affects melanopsin-containing ganglion cells and their dendritic structure are demonstrated based on the statistical analysis of the data collected. **Figure 1** and **Figure 2** show an example of a tracing for melanopsin retinal ganglion cells not affected by glaucoma and affected by glaucoma. **Table 1** presents all of the raw data collected from tracing. **Table 2** shows that all of the tested groups were normally distributed (passed the normality test) except for the normal soma size group. **Table 3** shows the comparison of normal soma size and glaucoma affected soma size to have a p-value of 0.0266. It also showed the

difference in median estimate as 1.7655. **Table 4** shows the comparison of normal total dendritic length and glaucoma affected total dendritic length to have a p-value of <0.0001. It also showed the mean sample difference between groups to be 364.62024 with a standard error of 63.602011. **Table 5** shows the comparison of normal dendritic field size and glaucoma affected dendritic field size to have a p-value of <0.0001. It also showed the mean sample difference between groups to be 12579.742 with a standard error of 2763.1552. **Table 6** shows the comparison of normal dendritic field diameter and glaucoma affected dendritic field diameter to have a p-value of <0.0001. It also showed the mean sample difference between groups to be 56.213338 with a standard error of 11.53696. The sample size was n = 20 for each group.

Discussion:

Soma size, total dendritic length, dendritic field size, and dendritic field diameter measurements were collected for mice retina's with and without glaucoma to see if glaucoma had a significant effect on dendritic structure of melanopsin retinal ganglion cells. The null hypothesis stated that there is no difference in the means between normal and glaucoma affected mice; the alternative hypothesis stated that there is a difference in the means between normal and glaucoma affected mice. In every statistical comparison between normal and glaucoma affected groups, the p-value was less than 0.05. This strongly indicates that the null hypothesis should be rejected. Meaning, there is a significant difference in the means between normal and glaucoma affected mice. In addition, the difference in median estimate (**Table 3)** as well as the mean sample differences (**Table 4-6**) for each group (normal minus glaucoma) were all positive values. This demonstrates that overall, glaucoma affected cell soma size, total dendritic length, dendritic field size, and dendritic field diameter, were smaller than normal cell soma size, total dendritic

length, dendritic field size, and dendritic field diameter. Glaucoma definitely has a considerable role in regards to the effect it has on melanopsin-containing retinal ganglion cells. However, it is still a large study point to continue research and further experiments to better explain and understand how this plays a role with circadian rhythm dysregulation and neurodegenerative diseases like Alzheimers.

Circadian rhythms are important in the eye when it comes to treating any problems within the eye as well as maintaining homeostasis (Martinez-Aguila et al., 2021). The system is within the suprachiasmatic nucleus (SCN) in the hypothalamus of the brain (Coogan et al., 2013). Melanopsin is a molecule that is involved in circadian rhythms - so when melanopsin ganglion cells are damaged due to glaucoma, there could be a problem in this cycle (Martinez-Aguila et al., 2021). However, there have been studies to show that even blind people could still have a regular circadian rhythm cycle, waking up and sleeping in sync with the environment (Do, 2019). The photoreceptors are needed primarily for the visual processing pathway, but aid in one's circadian rhythm cycle with the melanopsin. To explain how one could still have a functional circadian rhythm with damaged melanopsin-containing retinal ganglion cells could be that enough photoreceptors would be present to still bear simple functions, but just not bear the conscious perception aspect (Do, 2019). The severity of the damage in the melanopsin ganglion cells, drives the severity of dysregulation in circadian rhythms, which in turn play a huge role in neurodegenerative diseases like Alzheimers for example.

Alzheimer's disease is progressive neurodegenerative disease where memory and mental functions are impaired. When there are alterations in the SCN, it leads to disturbances in circadian rhythm cycles (Coogan et al., 2013). Studies have shown that in Alzheimer's patients, alterations in the SCN and disturbances in the circadian rhythm cycles are significant (Coogan et

al., 2013). Alzheimer patients have also been studied to generally have shorter dendrites (Einstein et al., 1994). Strong dendritic structure is important because they increase surface area in order to create a larger storage capacity for learning and memory (Wu et al., 2019). It was shown in the results that glaucoma had a significant effect on dendritic length, field size, and field diameter. Knowing that dendritic structure affects signaling, learning, and memory processes, it supports the literature that shorter dendritic structure is associated with Alzheimer disease and glaucoma is a factor in altering that (Wu et al., 2019).

Furthermore, the results of this experiment supported the hypothesis that there is a difference in the means between normal and glaucoma affected mice and that glaucoma has a significant effect on melanopsin-containing retinal ganglion cells. This study will help further support research behind glaucoma effects on circadian rhythms and Alzheimer disease. However, more experiments and more research can still be done to see how glaucoma affects other types of retinal ganglion cells - not just melanopsin-containing photoreceptors - neurodegenerative diseases, overall health, basic cognitive functions, and types of visions in the retina.

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