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Glaucoma Alters the Morphology of Mouse Melanopsin-Containing Retinal Ganglion Cells

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Abstract

The visual system is composed of various types of cells that all work in conjunction with one another to send and respond to stimuli from outside of the body. The retina in particular contains a concentration of cells that mediate vision, including a cell type known as the melanopsin ganglion cell that express the protein melanopsin. Melanopsin ganglion cells are intrinsically photosensitive, and are responsible for some aspects of non-image forming vision, such as maintaining circadian rhythms. Patients with glaucoma commonly have side effect symptoms associated with malfunctioning circadian rhythms, indicating a link between glaucoma and melanopsin ganglion cells. In this study, I traced melanopsin ganglion cells from mouse retinas with and without glaucoma at various pathological timepoints and took morphological measurements. These measurements were compared to see if glaucoma had an effect on melanopsin ganglion cells. The data collected showed that, of the morphological characteristics analyzed in this study, glaucoma has an effect only on dendritic field size. It appears that in retinas with glaucoma, melanopsin ganglion cells tend to have a larger dendritic field size than control cells; further research will help investigate why only this aspect is affected, and in the manner that it is.

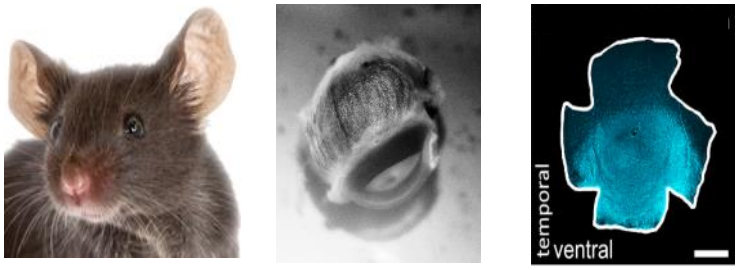
Introduction

Glaucoma is an eye disease that results in an abnormal increase in intraocular pressure that can result in damage to the optic nerve and in turn blindness; it is the second leading cause of blindness worldwide. Glaucoma is a chronic and progressive disease, and is marked by loss of peripheral vision and degeneration of optic nerve fibers and retinal ganglion cells, or RGCs (Obara et al 2016). An issue experienced by many patients with glaucoma is an alteration of their circadian rhythms. These circadian rhythm issues experienced by patients can cause difficulty sleeping, staying awake, waking up, and concentration as well as fatigue, stress, and even depression. Circadian rhythm disruption is seen in other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases and is accepted as a "natural consequence" of the diseases, but studies have shown that they may make the degeneration worse (Lax et al 2019).

Within the retina, there is a particular cell known as the melanopsin ganglion cell. These cells express the protein melanopsin, hence their name, coded by the Opn4 gene, and are considered a third type of photoreceptor (Sondereker et al 2020). Melanopsin ganglion cells are intrinsically photosensitive, meaning they respond to light without outside photoreceptor (rod or cone) input, and therefore mediate certain aspects of non-image forming vision. The most relevant of these aspects within the realm of vision is their important role in maintaining our circadian rhythms. Because glaucoma and melanopsin ganglion cells both have a connection to the regulation (and dysregulation) of circadian rhythms, this project investigates whether or not glaucoma has an effect on the morphology of these cells that may cause this defect. Because glaucoma causes dysregulation of circadian rhythms, it is hypothesized that the melanopsin ganglion cells will be smaller in size from pressure damage and therefore not able to perform their jobs adequately, resulting in these common issues with light sensing and circadian rhythms in patients. Previous studies have shown that ablation of M1 ipRGCs result in the loss of these non-image forming behaviors (Sondereker et al 2020).

Methods

DBA/2J mouse retinas from control and glaucomatous retinas at various pathological timepoints were dissected, fixed, and stained using immunohistochemistry. The DBA/2J mouse line is a well-accepted model as they are predisposed to spontaneous pigmentary glaucoma, characterized by intraocular pressure elevation and progressive death of retinal ganglion cells with age, closely resembling human glaucoma (Zhang et al 2013). This immunohistochemistry uses antibodies that tag the



The Jackson Laboratory | Sondereker et al 2018 | Sondereker et al 2018

Figure 1: Mouse retinas were transected and stained with antibodies that tag melanopsin protein. The retinas were then imaged and cells that expressed melanopsin would fluoresce. Scale bars = 1 mm. Mouse photo credit: The Jackson Laboratory

melanopsin protein. During imaging, the cells that express this protein will fluoresce green, allowing the mRGCs to be differentiated from other retinal cells. The retinas were then imaged using confocal imaging to create a z-stack of images that can be loaded into a specialized computer program known as ImageJ as a .tif file; each retina was divided into quadrants, so each .tif file is a quadrant of a particular retina with the melanopsin ganglion cells marked to be traced. ImageJ has a subprogram known as Simple Neurite Tracer that allows the user to automatically follow and digitally trace the dendrites of a single cell throughout the image stack, following and connecting the pixels to make a 2D image of how the dendrites stratify throughout the retinal quadrant.

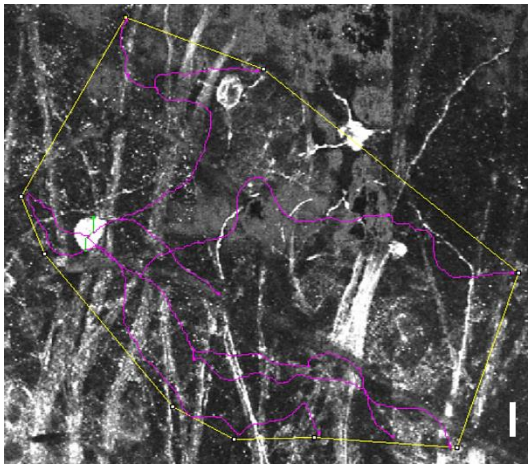


Figure 2. ipRGC of a 4-month glaucomatous mouse retina traced using ImageJ. Scale bar = 15 microns.

Once all of the dendrites on an individual melanopsin ganglion cell neuron are traced, ImageJ has various measurement features that allow for measurements to be taken of morphological characteristics such as soma size, total dendritic length, and total dendritic field size. This process of measurement was repeated with each traced cell in every quadrant until each melanopsin ganglion cell in a full retina is traced, and then the process was further repeated on another retina. This was repeated until enough data was obtained to compare between control and glaucomatous groups, as well as at different pathological timepoints such as early, mid, and late-stage glaucoma. As this is a major undertaking of data collection, data collected by multiple undergraduate students over the span of 4 years was analyzed for this project. This data was then statistically analyzed between groups using an ANOVA test with GraphPad InStat.

Results

Upon ANOVA analysis testing of the traces of 103 glaucomatous mouse retinal cells and 88 control mouse retinal cells, it was found that the differences in average soma size and total dendritic length were not significant. However, the differences in average dendritic field size between the two groups was significant. This significance was determined using the q value given as a result of the Tukey-Kramer Multiple comparisons test following the ANOVA. This indicates that glaucoma only affects certain morphological aspects of melanopsin ganglion cells; for this study, this is only in dendritic field size, which seems to be increased in glaucomatous retinas.

	Glaucomatous	**Control**
Sample Size (n=):	103	88
Soma Size:	**Glaucomatous**	**Control**
Mean (microns)	15.36	14.62
Standard Error (microns)	0.2684	0.2126
Tukey-Kramer Multiple Comparisons test, q value	0.000459	not significant
Total Dendritic Length:	**Glaucomatous**	**Control**
Mean (microns)	1358	1119
Standard Error (microns)	58.88	49.13
Tukey-Kramer Multiple Comparisons test, q value	0.1485	not significant
Dendritic Field Size:	**Glaucomatous**	**Control**
Mean (microns^2)	52746	44710
Standard Error (microns^2)	3501	2732
Tukey-Kramer Multiple Comparisons test, q value	4.982	significant

Table 1. Glaucomatous versus control melanopsin ganglion cell traces. This table analyzes the means, standard errors, and q values from the Tukey-Kramer Multiple Comparisons test for each variable (soma size, total dendritic length, and dendritic field size.) The q value must be over 4.03 to be considered significant based on the data entry. The only aspect that is significantly different between the two data sets is dendritic field size.

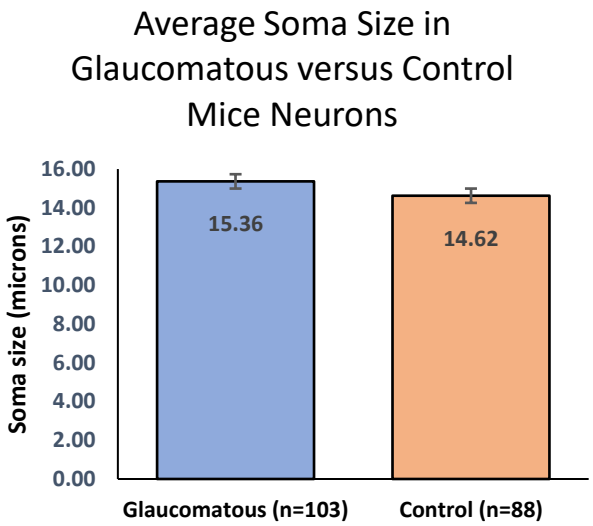


Figure 3. Average soma size as measured between glaucomatous and control mice melanopsin ganglion cell traces. There is no significant difference between the two data sets.

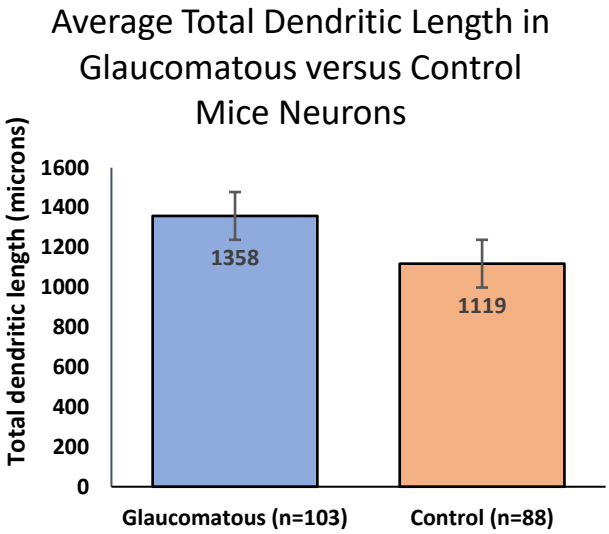
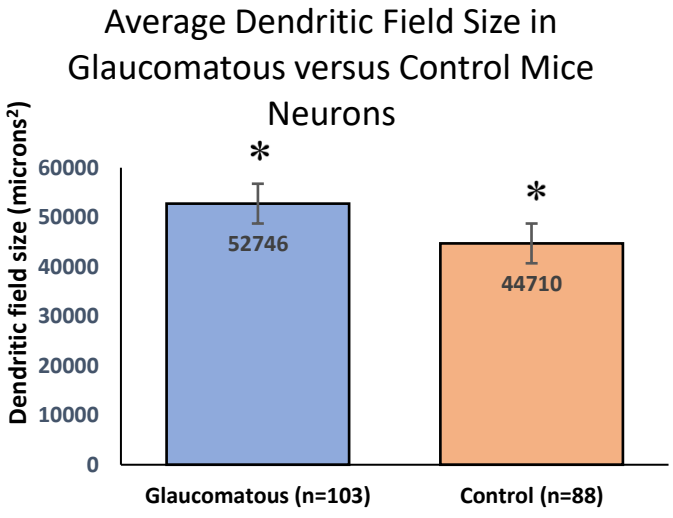


Figure 4. Average total dendritic length as measured between glaucomatous and control mice melanopsin ganglion cell traces. There is no significant difference between the two data sets.

Figure 5. Average total dendritic field size as measured between glaucomatous and control mice melanopsin ganglion cell traces. There is a significant difference between the two data sets, indicating that glaucoma has an effect on this aspect of morphology.



Discussion

After statistically analyzing a number of traces from glaucomatous and control mouse retinal ganglion cells, findings show that the only morphological aspect of these cells affected by glaucoma is dendritic field size. The original expectations of results were that perhaps the cells would become morphologically smaller from pressure damage, but it appears that this is not the case; soma size and dendritic length is the same between groups, but dendritic field size increases. This increase in field size but maintenance of dendritic length implies that on average there may be fewer dendrites found in glaucomatous cells, but they reach further than that of the controls for these numbers to alter in such a way. It is possible that a decrease in total dendrites (and potentially total number of cells in a glaucomatous retina) may cause an overcompensation of field size to be able to still fully span the retina. This would be an unsurprising find, as it is important to note that melanopsin ganglion cells have been found in various studies to be incredibly resistant to injury when compared to other retinal ganglion cells, so this could be another form of damage resistance (Lax et al 2019). A similar finding has come from studies with other rodent models, such as in a rat ocular hypertension model, where very few melanopsin ganglion cells degenerated compared to other RGCs (Li et al 2006). In particular, this injury resistance in specific regards to elevated intraocular pressure has been shown in the M1 subtype of melanopsin ganglion cell which was the focus of this study; results may differ in the M4 subtype, as they have been shown to suffer significant loss of cells with elevated IOP (Gao et al 2022). Therefore, the implication of this and other studies on the subject is likely not uniform among the different subtypes of melanopsin ganglion cells. It is, however, important to note some shortcomings of this study and others of its kind in terms of takeaway for human usage in early detection and treatment: human retinas are

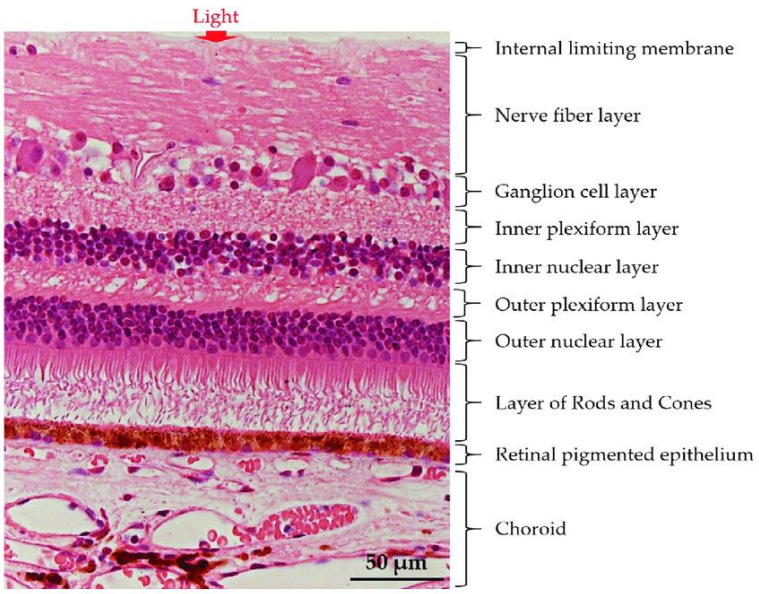


Figure 6. Histological arrangement of retinal structure. M1 and M1d somas can be found in the GCL and INL. Image from Triviño et al 2012.

fairly different in distribution of retinal ganglion cells compared to rodent models. Human retinas have melanopsin ganglion cells evenly distributed throughout the ganglion cell layer (GCL) and inner nuclear layer (INL), but these cells vary in shape and diameter from each other. The highest density of melanopsin ganglion cells were located around the fovea, an anatomical aspect that mice do not possess. M1 and displaced M1s are mainly concentrated in the temporal retina in humans (Hannibal et al 2017). In mice, they are asymmetrically distributed to the dorsal retina (Sondereker et al 2020) and in rats they are at a slightly higher density in the upper-temporal part of the retina (Lax et al 2019). It is important to take these anatomical differences into account when considering clinical implications.

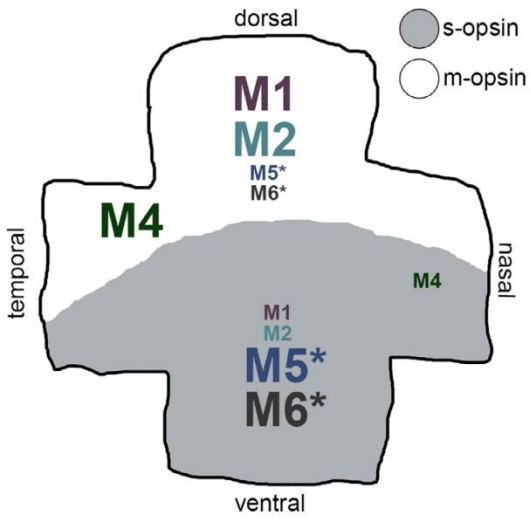


Figure 7. Distribution of melanopsin ganglion cell subtypes in the mouse retina. Image from Sondereker et al 2020.

In regards to the findings of this particular study, we can assume that the lack of morphological change of certain aspects of melanopsin ganglion cells can be attributed to the aforementioned resistance to injury. Further studies could be performed to investigate reasons for the increase in dendritic field size of glaucomatous cells, focusing more in depth on pathological timepoints or specific retinal quadrant. This study looked at the effect on the cells as a whole, so cells analyzed included the pathological timepoints of 3 months, 5 months, and 7 months, but did not take into account the potential difference of dendritic field size at each of these timepoints individually. It is likely that in the older retinas that have further progressed glaucoma, the more damage to the cells. This can be hypothesized off of the basis that in human eyes with glaucoma, studies have shown that a small decline in mRGCs occurs after the age of 50, marked by a stark loss of 44% after 70 years old (Lax et al 2019). In the same way, cells from temporal, dorsal, nasal, and ventral quadrants were analyzed all together; because of the difference in distribution patterns between models, it would be worthwhile to analyze cells from each quadrant separately to see if the distribution affects morphology as well in glaucomatous cells. It is evident that these cells have a special connection to this disease and others, and further studies will help uncover more attributes of their relationship.

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