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Understanding Multiple Sclerosis Through Retinal Cell Layer Thickness: An Insight into the Neurodegeneration Process

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Understanding Multiple Sclerosis Through Retinal Cell Layer Thickness: An Insight into the Neurodegeneration Process

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Abstract

Patients with multiple sclerosis (MS) have long been known to suffer deterioration of the retina, sometimes leading to blindness. The damage has been assumed to occur predominantly within the retinal nerve fiber layer. Using optical coherence tomography (OCT), researchers at Johns Hopkins University have determined that there is a subset of MS patients for whom the retinal deterioration is different. For this group the deterioration occurs at a deeper level within the retina, between the photoreceptors and the connecting cells. This deterioration affects the inner and outer nuclear layers of the retina. These findings are perplexing, as MS is considered primarily a demyelinating disease, and the retinal cell structures are unmyelinated.

Acknowledgements

I would like to express an immeasurable amount of gratitude to the research team at Johns Hopkins University for allowing me to work with them and their data. Without them, my thesis never would have passed the concept stage. I would also like to express my gratitude to Dr. Bruno Jedynak for introducing me to said team, and for all of his hard work in making this process run smoothly.

My greatest debt is owed to Dr. Jong Kim. Anytime I was uncertain how to proceed, his door was always open; he always answered every question with a smile. The completion of this project is a testament to his endless patience.

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Introduction

In the spring of 2013, each of my family members contracted a mild stomach virus. Each of us recovered in a couple of days, but then my mother relapsed. She became sicker as the days passed, and then, strangely, the right side of her body began to go numb. When the right side of her face developed a slight sag, we went into panic mode. We conferred with her doctor, and stopped at the lab for her to have a battery of blood tests on our way to the clinic. The doctor confirmed the obvious: something was very wrong. There was an emergency MRI, a follow-up MRI, an immediate hospitalization, a spinal tap, visits from neurologists, and finally, a diagnosis: multiple sclerosis. These two words changed the world for our entire family.

Multiple sclerosis is a neurological condition marked by inflammation resulting from autoimmune attacks to the myelin sheath on nerve cells in the brain and spinal cord. Once the myelin has sufficiently deteriorated, electrical impulses are no longer able to travel properly along the neurological pathways between cells. Demyelination appears as lesions on the brain and spine, and as white rings throughout the brain, both of which are visible on an MRI. It usually strikes young adults, and women more frequently than men. Symptoms include muscle spasticity, fatigue, bladder and bowel problems, motor dysfunction, cognitive difficulties, and vision issues (*Neurological Disorders*, p. 117). Patients' experiences can range from a complete lack of symptoms to severe symptoms, culminating in death (Zarelli).

The exact cause of MS is still uncertain. According to Kaiser Permanente MS specialist Dr. Greg Zarelli, neurologists generally agree that MS results from a combination of genetics, environment, and viral exposure—most likely to the Epstein-Barr virus. For many years, research has supported the likelihood that the disease is triggered by early viral infection and

subsequent autoimmune dysfunction (Bradshaw and Mattingley, p. 369). A child of an MS patient has a 1 in 25 chance of developing the condition, while the rest of the population has a 1 in 600 chance (Zarelli).

Researchers at Johns Hopkins University were kind enough to share data from ongoing research into the effects of MS on patients' vision, specifically the anatomical changes within the retina. While it is a very small region, with the help of optical coherence tomography (OCT), researchers can examine separate parts of the retina that would be invisible to the human eye. Without the OCT, the only way to see the differences being measured would be with the removal of the eye.

Patients with MS have long been known to suffer deterioration of the retina, sometimes leading to blindness. Until now, researchers believed that deterioration occurs in the cells connecting the retinal cells to the optic nerve (Saidha, *Primary Retinal Pathology*, p. 519). Demyelination has been thought to be the root cause of these vision issues, as of other MS symptoms. However, findings from ongoing research at Johns Hopkins University, using OCT, showed a subgroup of multiple sclerosis patients for whom the deterioration occurs at a deeper level within the retina, between the photoreceptors and the connecting cells (*Primary Retinal Pathology*, p. 524, 528). This atypical progression is perplexing, as MS is considered primarily a demyelinating disease, and the area of the eye structure affected in this subset has no myelin to be damaged and is distal to the optic nerve. In the larger sense, the findings based on this data challenge what has been our understanding of what MS is and does. That discovery formed the impetus for this paper.

It is impossible for readers to visualize the pertinent areas of deterioration in the retina without a basic understanding of the anatomy of the human eye. The retina forms a layer of tissue around the inside of the eyeball, anywhere that light could possibly reach inside the eye. Its function is to detect and interpret light, and then transmit the interpreted signals to the brain via the optic nerve (Dowling, p. 5). (See Figure 1)

Using electronics metaphors, the retina is like a DVD reader, picking up and transporting visual signals. The optic nerve is the cable between the DVD player and the television. Inner structures of the brain act as the TV, processing and combining signals to create the TV picture with 'sound' and 'color.' The visual cortex displays the entire TV picture, with all the final adjustments.

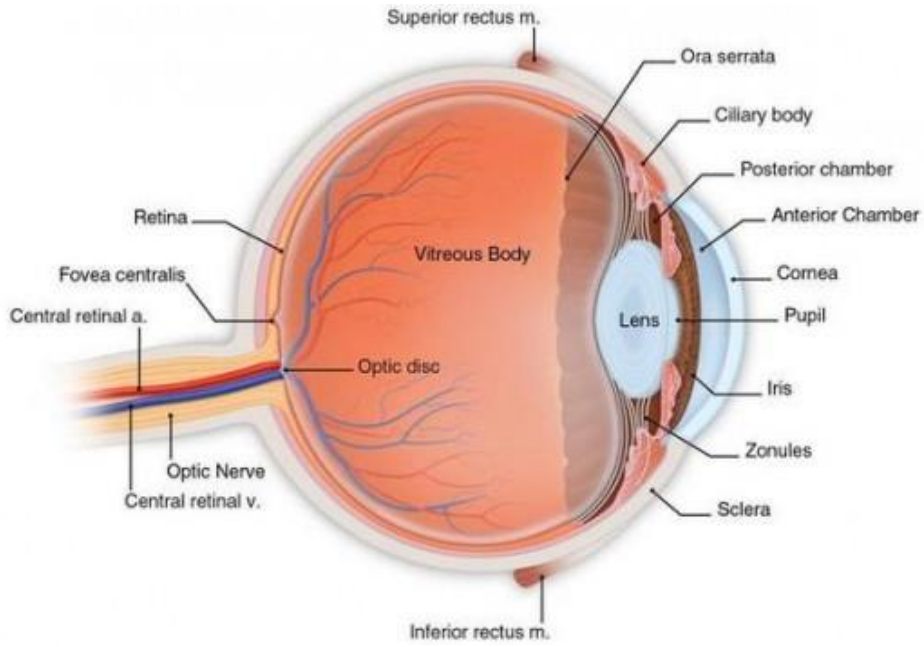

Figure 1: This image illustrates the structures of the eye. *Provided by B. Antony*.

In the eye, light enters through the pupil. It reaches the retina, where it is absorbed by the photoreceptors (rods and cones). From there, visual information signals are transmitted through "intermediary" neuron cells to ganglion cells. Long, unmyelinated axons connect the

ganglion cells to the optic nerve. All of the neuron cells in this circuit are segregated into three distinct layers; the fibers connecting the retinal neurons form two separating layers, while the axon fibers running to the optic nerve form a third layer (Dowling, p. 19).

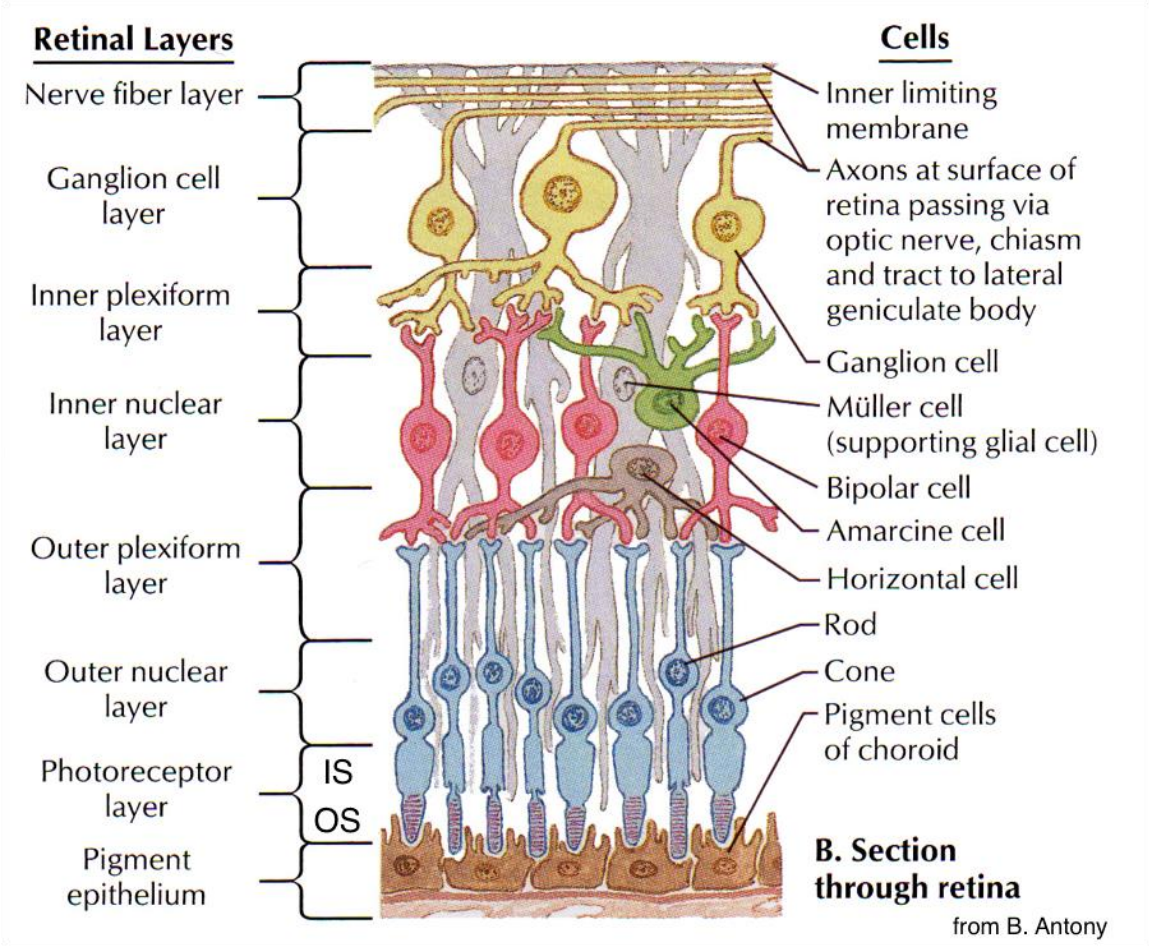

Figure 2: The retina consists of many layers. The retinal nerve fiber layer (RNFL) is the layer closest to the inside of the eye; the retinal pigment epithelium (RPE) is the outermost boundary of the retina. In this study, the photoreceptor layer was divided into two sections: inner segments (IS) and outer segments (OS), and are here labeled as such.

The outer nuclear layer (ONL) contains the nuclei and cell bodies of the photoreceptor cells. The cell bodies of 'intermediary' retinal neurons are found in the inner nuclear layer (INL). The synaptic connections between the two nuclear layers form the outer plexiform layer (OPL),

while the connections between the INL and the ganglion cells form the inner plexiform layer (IPL). The ganglion cell bodies are in their own layer, the ganglion cell layer (GC); the layer of axonal “nerve fibers” connecting the ganglion cells to the optic nerve is called the retinal nerve fiber layer (RNFL). Occasionally, cells may be found outside the typical location—for instance, ganglion cells have been observed within the inner nuclear layer (Dowling, p. 19).

A neuron has dendrites at one end, and an axon, coated by the myelin sheath, on the other. The axon has spaces (nodes of Ranvier) between segments of myelin that the nerve signals jump. In the peripheral nervous system (PNS), Schwann cells wrap around the axon to build the myelin sheath and clear away debris from their area of the axon; Schwann cells control only the one segment they comprise (Vargas, p. 160). In the central nervous system (CNS), instead of Schwann cells, the axon and its myelin sheath are controlled by oligodendrocytes. Picture an octopus, with each tentacle dedicated to one of the segments.

Herein lies the anomaly that is our current focus: the PNS, with its Schwann cells, regenerates myelin when damaged. Because each gap has its own support cells, lost areas of myelin can recover from damage. In the CNS, however, if the myelin is damaged, the debris is not cleared, and the oligodendrocyte does not regenerate (Vargas, p. 155). In the retina, neither the myelin nor the oligodendrocytes are present. And yet, there is still measurable degeneration. The degeneration is so unexpected and unaccountable that researchers were forced to draw the conclusion that they were observing some sub-type of MS retinal pathology that had not yet been identified.

These findings are significant because they raise the possibilities that there are other causes of MS than demyelination. The location of this degeneration is also important because

the retina is the easiest of the MS-degenerating regions to be observed in a living person. We may find that the retina, which is connected through the optic nerve and is part of the central nervous system, will make it easier to observe changes affecting the entire CNS. It is also important that, as 99% of deceased MS patients show these retinal changes to a significant extent, we can be certain they are related to the disease. That the retinal degeneration from this 'demyelinating' disease is also happening where there is no myelin frustrates explanation when we look at what MS reportedly does to cause degeneration. While it is widely accepted that there is much we do not know about this disease, there are indicators in this data that we know even less than formerly thought.

The findings at Johns Hopkins have met with some opposition from researchers. After Saidha, et al, published this research in the article, "Primary Retinal Pathology in Multiple Sclerosis as Detected by Optical Coherence Tomography," a German research team vehemently disagreed in a letter to the editors of *Brain*, the journal that had published the finding. The team of writers, Brandt et al, looked at the data from their own cohort and argued that, rather than a special subset, the findings could be explained by extreme cases of normal progression. The German team claimed that the grouping criteria defined by the researchers at Johns Hopkins created a significant difference where none actually existed.

Several inconsistencies are apparent when examining the Brandt argument. As the researchers at Johns Hopkins stated in their rebuttal, different scanners were used by the two research groups, which can cause noticeable variations in data. Secondly, in analyzing their data, the German research team used a scatter plot and linear regression analysis, while the Johns Hopkins team used standard T-testing. Finally, geographic location and nationality could be a

confounding variable as the frequency of MS has varied geographically (*Reply: Primary Retinal Pathology*). The Johns Hopkins team did not mention in its rebuttal another factor, specifically that the Germans compared total macular volume against the average RNFL thickness, whereas the original study used average overall macular thickness. The differences in method would almost certainly give differing results.

Methodology

Johns Hopkins University provided data for macular layer thickness, obtained using Spectralis OCT imaging and segmentation protocols. Technicians took retinal scans of 142 patients with relapsing-remitting multiple sclerosis (RRMS) and 114 control subjects. These formed the basis of the multiple sclerosis (MS) and healthy control (HC) subject groups. Some of the subjects from the HC were excluded if they had pre-existing conditions that would interfere with establishing a "normal" against which to compare patient data. Additionally, subjects from both the MS and the HC group were excluded if OCT data for only one eye was available; where more than one scan was provided for one eye, the first was kept (unless otherwise specified), and the extras were excluded from analysis.

After exclusions, there were 133 subjects in the MS group and 96 subjects in the HC group. The average of left and right eye data was subsequently determined for each subject and used for analysis. Following criteria laid out in "Primary Retinal Pathology" (p. 519-20), the MS group was divided into three smaller groups for analysis. The original criteria compared MS patient data against a database of controls, setting "normal" values as between the 5^{th} and 95^{th} percentiles of the control database and "below normal" values as less than the 5^{th} percentile (*Primary Retinal Pathology*, p. 522). Group 1, labeled "macular thinning predominant" (MTP),

contained subjects demonstrating “below normal” macular thickness and “normal” RNFL thickness. Group 3, labeled MSN for “multiple sclerosis – normal scans”, contained subjects demonstrating “normal” RNFL and average macular thicknesses. Group 4, labeled HC for “healthy controls”, contained the set of healthy subjects exhibiting no symptoms of optic deterioration. Group 2 contained MS patients demonstrating the “abnormalities typical of those seen in multiple sclerosis—‘below normal’ RNFL thicknesses on OCT, regardless of macular thinning.” The group was labeled MSA for “multiple sclerosis – abnormal scans”. In this thesis, these grouping criteria were partially replicated; in place of the database, the percentiles were defined exclusively using the HC data provided. According to the Johns Hopkins team, no comparable database currently exists.

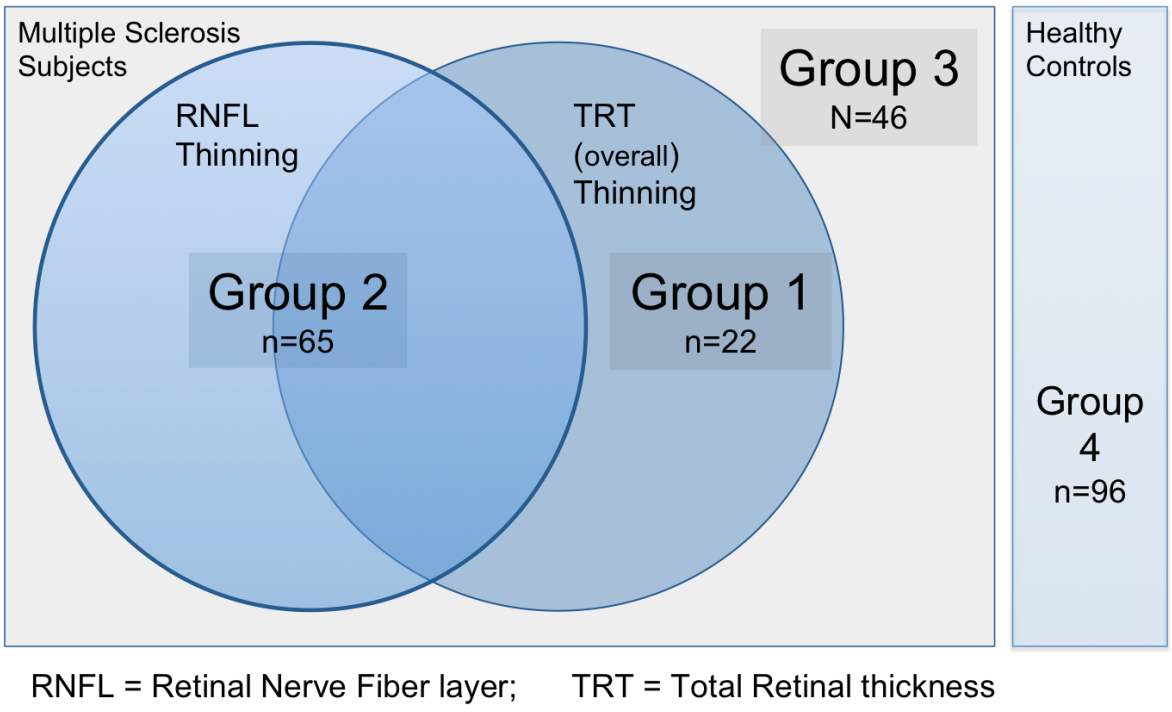

Figure 3: The Venn diagram above illustrates the grouping criteria at work.

The Venn diagram in Figure 3 helps visualize the groupings. Group 1 is patients with overall macular thinning without thinning of the RNFL. Group 2 is all patients exhibiting thinning of the RFNL, whether or not they have overall thinning. Group 3 patients don't show thinning in any layer. Group 4, seeming to be unconnected to the others, is the control group, people who do not have MS or any other preexisting condition that could affect the retina.

Wilcoxon testing was used to compare average thicknesses of each layer between groups, and a p-value of less than 0.001 was considered significant. Since we used an alpha of 0.001 for each comparison, by Bonferroni's inequality $[1- (1-\alpha)^{(\#\text{ of comparisons})}]$, the overall significance level of these tests is bounded above by 0.054. If we had used an alpha of 0.0001, we could have made an overall significance level bounded above by less than 1%. However, the individual alpha would have been very small, which means we might not have detected a difference where one actually exists.

Results

MTP and MSA demonstrated significant thinning in the RNFL compared to MSN and to HC ($p< .001$ for all). RNFL was significantly thinner in MSA compared to MTP, as well ($p < .001$). MSN was not significantly different from HC in RNFL or overall macular thickness. The GC-IPL of MTP and MSA subjects were not significantly different; however, both were significantly thinner than MSN, and all MS subjects had significantly thinner GC-IPLs compared to HC ($p< .001$).

Wilcoxon Test Results	Grp 1 vs Grp 2	Grp 1 vs Grp 3	Grp 1 vs Grp 4	Grp 2 vs Grp 3	Grp 2 vs Grp 4	Grp 3 vs Grp 4
RNFL	***p < .001***	p < .001	p < .001	p < .001	p < .001	p = 0.5518
GC-IPL	p = 0.3768	p < .001	p < .001	p < .001	p < .001	p < .001
INL	p < .001	p < .001	p < .001	p = 0.6004	p = 0.0373	p = 0.0801
OPL	p = 0.0024	p < .001	p = 0.0060	p = 0.7579	p = 0.0846	p = 0.0144
ONL	p < .001	p < .001	p < .001	p = 0.3519	p = 0.9739	p = 0.2965
IS	p < .001	p < .001	p < .001	p = 0.0800	p = 0.0530	p = 0.8122
OS	p = 0.0095	p = 0.0025	p = 0.0017	p = 0.4347	p = 0.8295	p = 0.4521
RPE	p = 0.0417	p = 0.0153	p = 0.0798	p = 0.4893	p = 0.5295	p = 0.1572
TRT	p = 0.0257	p < .001	p < .001	p < .001	p < .001	p = 0.5933

Figure 4 The table above contains the test results for each individual Wilcoxon test. All results with a p-value less than 0.001 are reported as such. Grp 1 = MTP, Grp 2 = MSA, Grp 3 = MSN, Grp 4 = HC.

The INL in MTP was significantly thinner than MSA, MSN, and HC ($p < .001$). Neither MSA nor MSN demonstrated significant differences in the INL against HC or each other. The OPL was thinner in MTP compared to MSA ($p = .002$), MSN ($p < .001$), and HC ($p = .006$), as well, without significant differences among the three. The ONL and IS layer were similarly significantly thinner in MTP compared to the other three groups ($p < .001$ for MTP vs all). There is evidence to support a claim of significant difference in the OS layer as well: MTP against MSA ($p = .010$), MSN ($p = .003$), and HC ($p = .002$), with little to no difference among non-MTP groups. No significant results were found within the RPE.

Analysis

The MTP group displayed significant thinning of the inner nuclear and outer nuclear layers, which was not found in either of the non-MTP MS groups. This supports the findings of the earlier Johns Hopkins University study, and suggests a previously unrecognized pathology in retinal neurodegeneration. However, at this time, it cannot be definitively claimed that this loss is not as a result of retrograde degeneration of the RNFL and subsequent neurodegeneration of

the retina—the accepted explanation before now. So, while it needs further support, it appears that degeneration does not stop at the first two layers, as has previously been posited (*Primary Retinal Pathology*, p. 208). While the damage to those two layers makes sense, given the presence of myelin in the optic nerve, the severity of degeneration present in the deeper retinal layers does not. As there is no myelin, there must be another explanation for the damage.

Furthermore, the MTP group displayed significant thinning in the inner portion of the photoreceptor segments (IS). Evidence suggests less significant thinning observed within the OPL as well, although the observation may be attributed to chance. Together with the ONL thinning, however, there is ample evidence to suggest that the photoreceptor cells, which compose much of the outer layers, are somehow involved with the observed thinning present in the MTP group. Further research may be enhanced by a concentration on this region in the retina.

While the research is highly suggestive, there are design issues to be addressed: database and experimental design. The database does not exist; while a database was used for the grouping criteria in the original study, the comparison control group was used as an alternative in this study. This weakens our ability to generalize our findings. Further design issues include a too-small pool of patients—only about half—with corresponding EDSS scores, and averaging measurements may have reduced sensitivity.

Conclusion

There is clear support for a new subset of MS patients with retinal damage beyond the first two layers. The etiology remains unclear. This conclusion agrees with the findings of Saidha's earlier study, as was anticipated.

One of Saidha's concerns was that grouping criteria are based only on RNFL and overall macular thickness among MS patients, and those experiencing retrograde degeneration as well as the degeneration described in the MTP group fail to be recognized. This appears to be a valid concern, and further criteria based around the deeper layers must be developed.

An interesting question remains: What exactly is happening deep in the retina where degeneration is occurring? Why is there degeneration where there is no myelin, under the normally affected retinal layers, when those layers may not show any degeneration? What is the process that is occurring that seems to have nothing to do with the demyelination that defines MS?

In the retina, where there is no myelin, we still find glial cells. Though oligodendrocytes are glial cells found throughout the central nervous system, they are completely absent from the retina. So why in the retina is there a parallel cell, an octopus like the oligodendrocytes, where there is no octopus work to be done? It suggests that the structures of the parallel cell have some function beyond maintaining and controlling the myelin sheath. We can make many speculations. For example, that function might have something to do with the blood-brain barrier, or amplification of nerve impulses, or the macrophagic (immune) system, or even something else that has not yet been examined. We do not yet know. The findings merit more research, likely with exciting results.

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Appendix

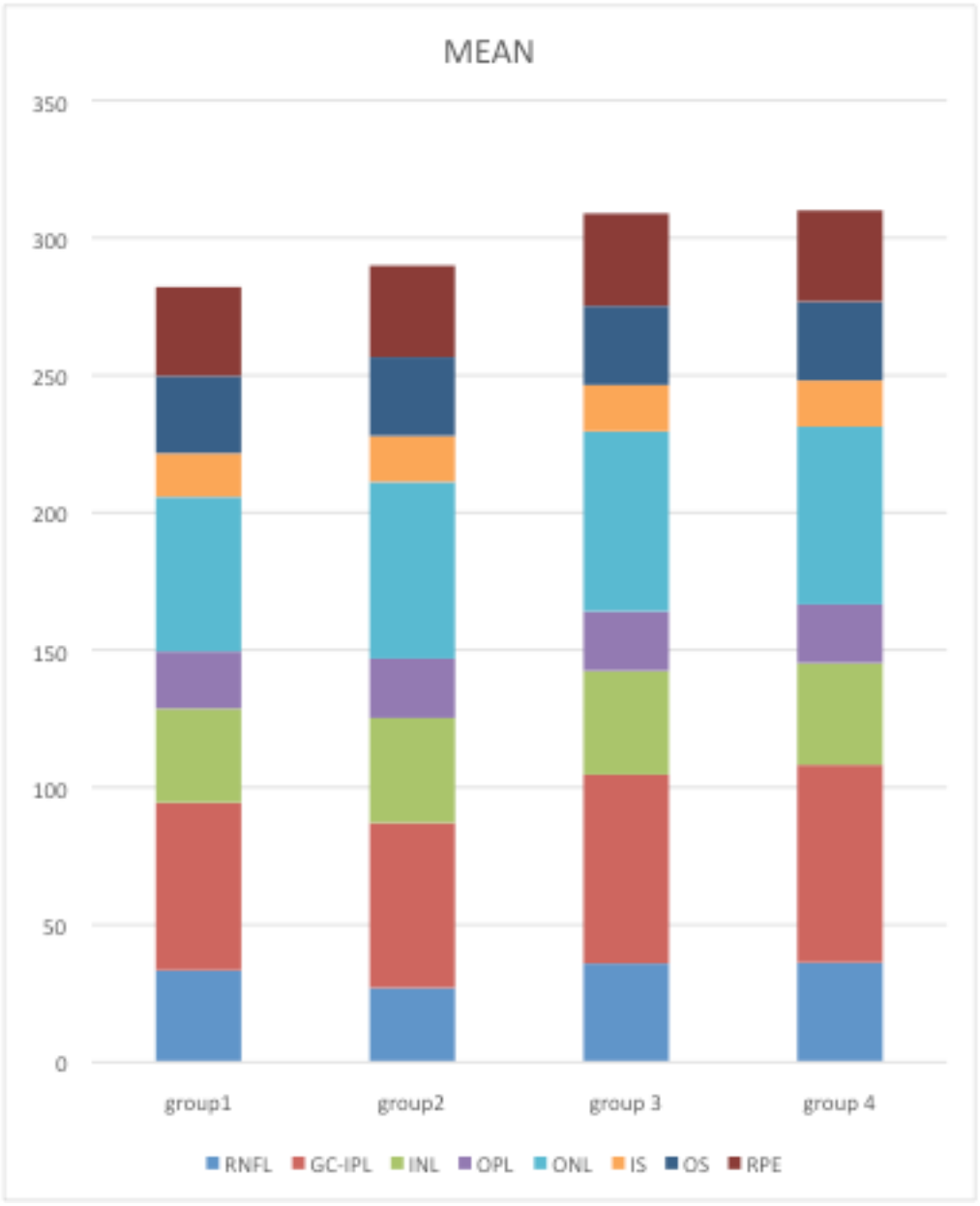

Figure 5 A chart illustrating the average thicknesses of each layer, and overall, for each group.