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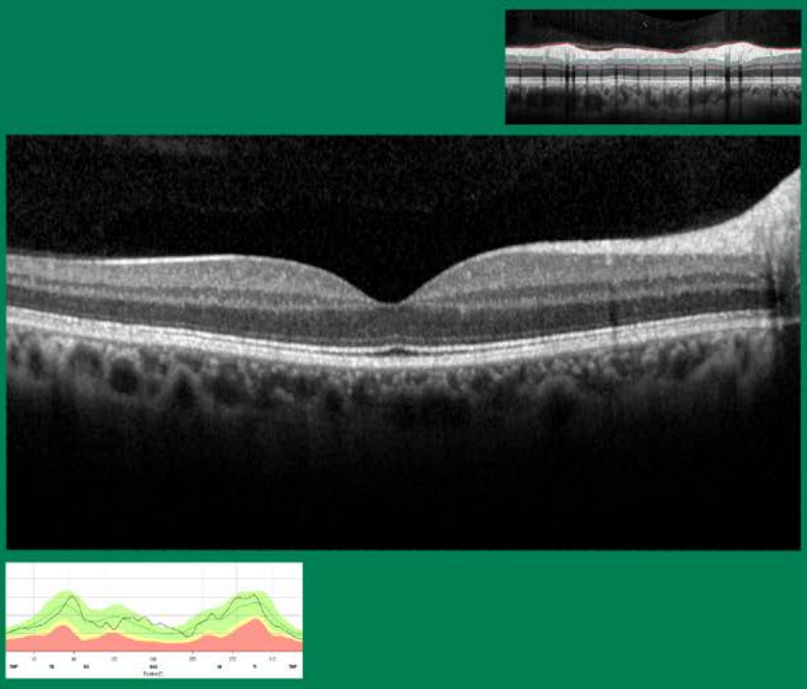
Retinal ganglion cell examination with Optical Coherence Tomography reflects physiological and pathological changes in the eye and the brain

Abbas Al-Hawasi



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Department of Biomedical and Clinical Sciences (BKV),
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Cover illustration: design and picture composition by Abbas Al-Hawasi. Upper picture is the OCT B-scan of the peripapillary retinal nerve fiber layer, middle picture is the B-scan of the macular retinal layers, lower picture is the thickness measurements of the peripapillary retinal nerve fiber layer.

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Retinal ganglion cell examination with Optical Coherence Tomography reflects physiological and pathological changes in the eye and the brain.

ACADEMIC THESIS BY Abbas Al-Hawasi

FOR THE AWARD OF A DOCTORATE DEGREE (Ph.D.)

BY

Linköping University,
Department of Biomedical and Clinical Sciences (BKV).
Division of Sensory Organs and Communication (SOK)

Faculty of Medicine, Linköping, Sweden

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To My lovely wife Deema
And
To my sons Zain and Zaid
For their endless support and love

The learner must persevere in seeking knowledge, never tire of seeking it, and not overdo what he has learned.

Ali Bin Abi-Talib 599-661 a.c.

Abstract

The retinal ganglion cell is situated in the inner retina and its axons, composing the retinal nerve fiber layer (RNFL), leave the eye to form the optic nerve. These cells develop embryologically from the forebrain and later during development re-establish connections with different parts of the brain serving different purposes. This unique position and connections make it possible to be investigated with different methods. Optical Coherence Tomography (OCT) is an accessible and easily operated clinical device that can provide a detailed image of this layer at a few micrometers level of precision in measurements. In this thesis we aimed to see whether examining these cells with OCT could reflect physiological and pathological changes in the eye and brain.

In cases of optic neuritis (Paper I), the OCT examination showed early thickening of the peripapillary (pRNFL) followed by thinning which takes 6-9 months to reduce to below normal thickness without the ability to distinguish between the real from pseudo thinning. The ganglion cell -inner plexiform layer (GCL-IPL) layer, however, showed a thickness reduction within a few weeks to 3 months without pseudo thinning.

In cases of Idiopathic Intracranial Hypertension (IIH) (Paper II), the GCL-IPL remained unchanged and there was no difference in pRNFL thickness compared to healthy controls, whereas the optic disc parameters of rim thickness, rim area, cup volume and cup/disc ratio differed significantly ($P<0.05$).

In cases of benign multiple sclerosis (Paper IV), the OCT could detect that eyes which are not affected by optic neuritis had an annual thinning rate of the RNFL and GCL-IPL similar to a healthy population ($P>0.05$) which may indicate the benign course of the disease.

In cases of physiological factors affecting the GCL in healthy population (Paper III) the OCT examination showed that there was a significant thinning rate of the layer with

age ($P<0.05$), but the thinning was not significant when sex and axial length of the eye were taken into consideration. Males had a thicker GCL volume than females and with age a significant reduction in GCL volume was noted in females but not in males. A Longer axial length of the eye found to be associated with thinner GCL volume.

In conclusion retinal ganglion cell changes detected with OCT can reflect physiological and pathological changes in the eye and brain.

Populärvetenskapliga sammanfattning:

Den retinala ganglioncellen är belägen i den inre näthinnan och dess axoner, näthinnans nervfiberskikt (RNFL) eller nervfibrer, lämnar ögat för att bilda synnerven. Dessa ganglionceller utvecklas under fosterstadiet från framhjärnan och återskapar senare under utvecklingen förbindelser med olika delar av hjärnan som tjänar olika syften bland annat synen. Denna unika position och kopplingar gör det möjligt att undersöka dem med olika metoder. Optical Coherence Tomography (OCT) är en tillgänglig apparat på de flesta ögonkliniker och används vid olika ögonsjukdomar som grönstarr och sjukdomar i gula fläcken. Apparaten är lättmanövrerad, kostnadseffektiv och undersökningen kan göras i samband med kliniska besök. Undersökningen kan ge en detaljerad bild av näthinnas olika lager med en mikrometers precisionsnivå vid mätningar. I detta projekt har vi syftat till att se om undersökning av gangliecellerna med OCT kunde återspegla fysiologiska och patologiska förändringar i ögat och hjärnan.

I fall av synnervsinflammation (Arbete I), visar OCT-undersökningen tidig förtjockning av nervfibrerna följt av uttunning, det tog 6–9 månader för att minska till under den normala tjockleken utan att kunna skilja mellan verklig och falsk förtunning. Gangliecell-skiktet däremot visade en tjockleksminskning redan inom några veckor till 3 månader. Detta faktum ger ett snabbare och mer pålitligt resultat.

I fall av tryck på synnerven på grund av ökat tryck i hjärnan, (idiopatisk Intrakraniell hypertoni) (Arbete II), förblir gangliecellslagret oförändrat och det finns ingen skillnad i nervfibrernas tjocklek jämfört med friska kontroller medan synnervshuvudets parameter som rim tjocklek och area, exkavationsvolymen och exkavation/synnervhuvudets-förhållande skiljer sig signifikant. Det bevisar att vid

denna sjukdom, synnervshuvudet är först att bli påverkat medan gangliecell lagret visade ingen direkt eller bestående skada på nerven.

Vid Multipel skleros drabbas patienterna av sjukdomen ofta successivt och man behöver sätta in behandling för att förhindra funktionshinder. En grupp av dessa patienter slipper uppenbara funktionshinder även efter flera år av sjukdomen trots uteblivet behandlingsbehov. Den typen kallas för godartad eller benign (BMS).

I fall av godartad multipel skleros (Arbete IV) kan OCT undersökningen upptäcka och visa att ögonen som inte är drabbade av inflammation på synnerven har en årlig förtunning som liknar den hos en frisk population vilket är mindre uttalad än hos dem med MS med svårare sjukdomsförlopp. Med hjälp av undersökningen kan man bevisa ett mer godartat sjukdomsförlopp hos MS patienter och möjligen inget behov till behandling.

I fall av fysiologiska faktorer som påverkar GCL i frisk population (Arbete III) visar OCT-undersökningen att det finns en signifikant förtunning av lagret med åldern men förtunningen är inte statistiskt signifikant när kön och axiell längd tas i beaktande. Män har en större gangliecell-volym än kvinnor och med åldern är det en signifikant minskning av volymen hos kvinnor, medan hos männen uteblir en signifikant förändring. Samma mönster ses i hjärnstorlek både med åldern och hos båda könen. En ökad axiell längd är associerad med tunnare ganglioncellskiktsvolym.

Sammanfattningsvis, en undersökning av retinala ganglieceller med OCT kan återspegla fysiologiska och patologiska förändringar i ögat och hjärnan och borde användas oftare för undersökning av patienter med olika sjukdomar.

List of Publications:

Paper I

Huang-Link YM, Al-Hawasi A, Lindehammar H. Acute optic neuritis: retinal ganglion cell loss precedes retinal nerve fiber thinning. Neurol Sci. 2015 Apr;36(4):617-20. doi: 10.1007/s10072-014-1982-3. Epub 2014 Oct 14. PMID: 25311917.

Paper II

Huang-Link YM, Al-Hawasi A, Oberwahrenbrock T, Jin YP. OCT measurements of optic nerve head changes in idiopathic intracranial hypertension. Clin Neurol Neurosurg. 2015 Mar; 130:122-7. doi: 10.1016/j.clineuro.2014.12.021. Epub 2015 Jan 7. PMID: 25614195.

Paper III

Al-Hawasi, A., Lagali, N. Retinal ganglion cell layer thickness and volume measured by OCT changes with age, sex, and axial length in a healthy population. BMC Ophthalmol22, 278 (2022). https://doi.org/10.1186/s12886-022-02488-7.

Paper IV

Al-Hawasi A, Lagali N, Fagerholm P, Huang-Link Y. Longitudinal Optical Coherence Tomography Measurement of Retinal Ganglion Cell and Nerve Fiber Layer to Assess Benign Course in Multiple Sclerosis. J Clin Med. 2023 Mar 14;12(6):2240. doi: 10.3390/jcm12062240. PMID: 36983241; PMCID: PMC10054631.

Related publications not included in the thesis.

Huang-Link YM, Petré B, Lindehammar H, Al-Hawasi A, Link H (2015) Homonymous Hemimacular Ganglion Cell Layer Loss Detectable by SD-OCT: A Biomarker of Retrochiasmal Visual Pathway Lesion. JSM Biomar 2(1): 1006.

Huang-Link YM, Al-Hawasi A, Eveman I. Retrograde degeneration of visual pathway: hemimacular thinning of retinal ganglion cell layer in progressive and active multiple sclerosis. J Neurol. 2014 Dec;261(12):2453-6. doi: 10.1007/s00415-014-7538-x. Epub 2014 Oct 14. PMID: 25311572.

Abbreviations

AMD	Age-Related Macular Degenration
AO	Adaptive Optics
Atoh7	Atonal BHLH Transcription Factor 7
Aβ	amyloid-Beta proteins
BHLH	Basic Helix-Loop-Helix
BMP	Bone Morphogenic Kinase
BMS	Benign Multiple Sclerosis
CSF	Cerebro-Spinal Fluid
cSLO	Confocal Scanning Laser Ophthalmoscopy
CT-scan	Computerized Tomography scan
CVA	Cerebo-Vascular Accident
DM	Diabetes Mellitus
DOA	Dominant Optic Atrophy
DTN	Dorsal Terminal Nucleus
ETDRS	Early Treatment Diabetic Retinopathy Study
EWN	Edinger-Westfall Nucleus
FAZ	Foveal Avascular Zone
FDT	Frequency Doubling techonology
GC	Ganglion Cell
GCL-IPL	Ganglion Cell layer-Inner Plexiform Layer
GCL	Ganglion Cell layer
HIF-1α	Hypoxia Inducible Factor 1 Alpha
HRR	Hardy Rand and Rittler plates
IIH	Idiopathic Intracranial Hypertension
IOP	Intra Ocular Pressure
IPL	Inner Plexiform Layer
ipRGC	Intrinsically photosensetive Retinal Ganglion Cell
Klf	Krüppel-like Factor
LGB	Lateral Geniculate Body
LGN	Lateral Geniculate Nucleus
LHON	Leber Hereditary Optic Neuropathy
LP	Lumber Puncture
LTN	Lateral Terminal nuclei
MAP kinase-FGF	Mitogen-Activated Protein-Fibroblast growth factor
Math	Mouse atonal BHLH
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MTN	Medial Terminal nuclei
NOT	Nucleus of Optic Tract
OCT-A, OCTA	Optical Coherence Tomography Angiography
OCT	Optical Coherence Tomography
ONH	Optic Nerve Head

ONL	Outer Nuclear Layer
OPN	Olivary Pretectal Nucleus
PAX	Paired box protein (gene)
pRNFL	Peripapillary Retinal Nerve Fiber layer
RGC	Retinal Ganglion Cell
RGCs	Retinal Ganglion Cells
RNFL	Retinal Nerve Fiber Layer
ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
SAP	Static Automated Perimetry
SCN	Supra Chiasmatic Nucleus
SD	Spectral Domain
SLD	Superluminescent Diode
SS	Swept Source
TD	Time Domain
TF	Transcription Factor
VEGF	Vascular Endothelial Growth Factor
VEP	Visual Evoked Potential

Table of Contests

1. Background

1.1 Retinal ganglion cell

1.1.1 Embryology of the brain and eye

Early in fetal development, there are three distinct layers of cells called the primary cell layers which consists of:

1. Endoderm (the inner layer) which gives rise to the respiratory and gastrointestinal system.
2. Mesoderm (middle layer) which gives rise to the skeleton, muscles, and connective tissues.
3. Ectoderm (outer layer) which is divided into:
   a. Surface ectoderm: which gives rise to the skin.
   b. Neural ectoderm: which gives rise to the central and peripheral nervous system.

The neural ectoderm originated from the ectoderm and its formation is regarded as the earliest step in the development of the nervous system (Gary C. Schoenwolf 2015).

Along the dorsal side of the embryo a group of ectodermal cells differentiate into neuro-epithelial cells and form the neural plate by thickening and migrating toward the midline of the embryo. These cells, later, undergo thickening and shape changing, causing the tissue at this area to invaginate later, to form the neural folds and tube. At the cranial end of the neural tube develops the brain. This invagination forms a groove-like structure along the dorsal surface of the embryo and is called the neural groove. The margins of the groove thicken and elevate to form a ridge-like structure on both sides of the groove and at this level the new structure is called the neural fold. As the margins of the neural fold converges toward the midline and comes together it forms the neural tube, just beneath the ectoderm.

This hollow tube represents the future nervous system in which the cranial part develops into the brain and the caudal part into the spinal cord.

Optic vesicles

The optic primordium appears on day 22 after fertilization in the neural folds and at the area of the future forebrain. Derived from the neuroectoderm, two optic pits appear on each side of the midline, which form the optic vesicles later (Figure 1)(BCSC 2021). The evagination of the optic vesicles is considered the first sign of ocular morphogenesis, which occurs during the final stages of neural tube formation in the forebrain's ventral part. There is evidence that these changes occur due to coordinated changes in cellular behavior and changes in cell shape as noticed in fish and frogs, as retinal progenitor cells modulate their convergence while medial retinal progenitor cells remain stationary (Rembold et al. 2006). One of the major molecular mediators that may play a cardinal role in the optic vesicle development is the retinal homeodomain transcription factor RAX, as it has been shown that defects in its alleles leads to anophthalmia as well as brain anomalies in the human, fish, and frog (Abouzeid et al. 2012) (Furukawa et al. 1997) (Kennedy et al. 2004) (Andreazzoli et al. 1999).

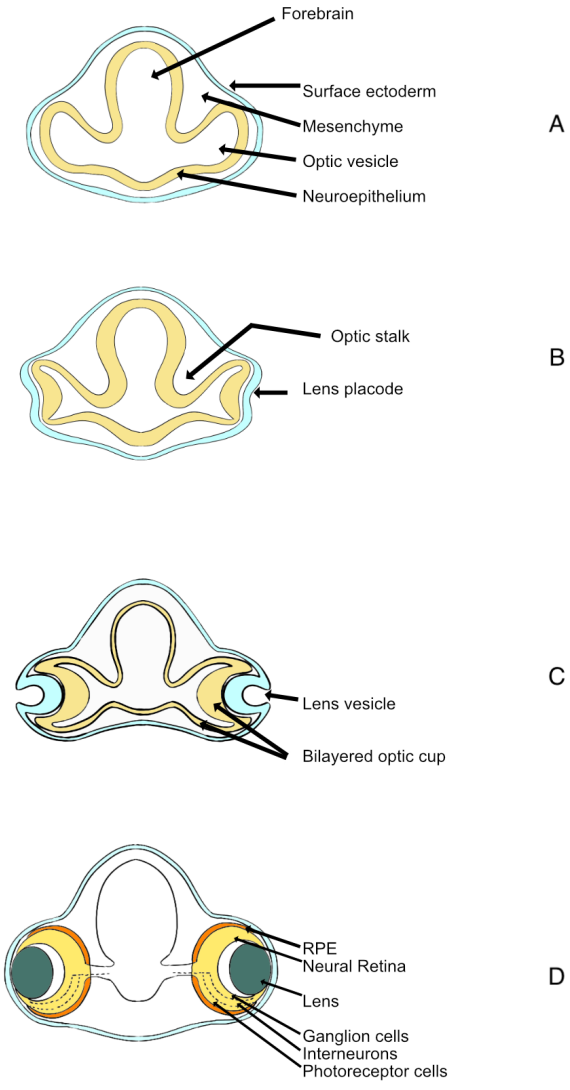


Figure 1. Early formation of the forebrain, optic vesicle, optic stalk, and development of the retina and retinal layers including the ganglion cell layer (by Deema Al-Hamdani).

During this process, the two-layered vesicle consists of an outer (dorsal) portion or layer which becomes the retinal pigment epithelium and a distal (ventral) portion from which the neural retina develops (Fuhrmann 2010) (Hirashima et al. 2008; Kagiyama

et al. 2005). In case of removal of the dorsal layer (which makes the retinal pigment epithelium) it could be replaced by the inner ventral layer. While in case of removal of the inner layer, the remaining outer layer can produce retinal pigment epithelium-like vesicle that fails to invaginate (Hirashima et al. 2008). The inner layer of the optic vesicle is called the neuroblast layer. The connection between the optic vesicles and the developing forebrain narrows and represents the future optic nerve.

Development of the sensory retina

Various stages of the formation of the neural retina depend on the Mitogen-Activated Protein Kinase-Fibroblast growth factor (MAP kinase-FGF) signaling pathway which is required for human deep-layer corticogenesis also (Gantner et al. 2021). This pathway is crucial for two processes: the start of retinal neurogenesis and the patterning of the retina in the distal optic vesicle. In chick embryos, the removal of the surface ectoderm prevents the expression of neural markers in the distal region (Fuhrmann 2010). Additionally, the patterning of the optic vesicle into the retina and RPE (Retinal Pigment Epithelium) is disrupted, as evidenced by the development of the eyes as pigmented vesicles with a few interspersed neuronal cells.

Different labeling methods by cytoplasm tracers or by retrovirus encoding of retinal progenitors showed that cells in the future sensory retina are pluripotent retinal progenitor cells with the ability to develop into many different retinal neuronal cell types as well as Müller glial cells. On the other hand, retinal astrocytes immigrate into the retina from cells in the optic stalk (Ling and Stone 1988) (Stone and Dreher 1987). Another signaling pathway that may play a significant role in early steps of the retinal development is the bone morphogenetic proteins (BMP) in which inactivation of its Ia

and Ib receptors leads to failure of the retinal neurogenesis initiation (Murali et al. 2005).

These pluripotent retinal progenitor cells give rise to different copies, from a few to a hundred copies per clone (Turner et al. 1990). The types of cells that are produced depend on the age when the progenitor leaves the cell cycle, in other words it depends on the cell's birthday (The Visual Neuroscience 2004, p.77-78). This fact is important but not absolute as there is also, to some extent, a spatio-temporal overlap. For example, cones are produced during the whole retinal neurogenesis process while ganglion cells are the earliest to leave the cell cycle. In temporal order after the ganglion cells follows the horizontal cells and cones while other cell types as amacrine, rods, bipolar and Muller glial cells develop progressively later. The fate of the retinal progenitor cells is a complex process, and it is unclear if it is an intrinsic strict order by the progenitor cell or there are some local environmental factors around the cell that determine its fate but most evidence points toward a combination of both.

Development of the retinal ganglion cell

The embryonic development of retinal ganglion cells involves a complex interplay of many factors that affect its development and later maturation both in temporal (time during the development) and spatial (place in retina).

Factors essential for retinal ganglion cell development

The differentiation and maturation of retinal ganglion cells from retinal progenitor cells (RPCs) is affected and regulated by intrinsic (intracellular) factors like genes, transcription factors and proteins as well as extrinsic (extracellular) factors. Many of these factors are also required for the development of the forebrain. These factors interact in a complex way in the form of feedforward and feedback signaling during various stages of the development of RGCs (Retinal ganglion cells) from transitioning from the retinal progenitor cells to RGC precursors, to become specialized and later to become diverse types of RGCs. Even arborization and axon development which it means the optic nerve formation is affected by such factors. Some factors that have a significant role in the development of the eye and ganglion cells are Pax6 (Halder et al. 1998) (Walther and Gruss 1991), Lhx2 and Lhx9 (Chou and Tole 2019), Math5(Atoh7) (Brown et al. 2001) (Prasov et al. 2012) (Gan et al. 1999), Wnt/β-catenin (Musada et al. 2020), Brn-3 (Pou4) Transcription factors (Jain et al. 2012; Pan et al. 2008), Notch-Ligand pathway (Nelson et al. 2006) (Jadhav et al. 2006; Nelson et al. 2007) and zinc-finger factor family (Krüppel-like) Klf7 and Klf4 (Laub et al. 2005).

Spatial RGC development and retina layering

The inner layer of the optic vesicle is the source of all retinal progenitor cells that develop into sensory retinal cells such as photoreceptors, bipolar cells, horizontal, amacrine and ganglion cells.

Mitosis of retinal progenitor cells occurs at the outer retina near to the retinal pigment epithelial layer (Figure 2). After mitosis and when the fate of the retinal cell is decided the nucleus of the developing cell migrates to the future somal destination (McLoon and Barnes 1989).

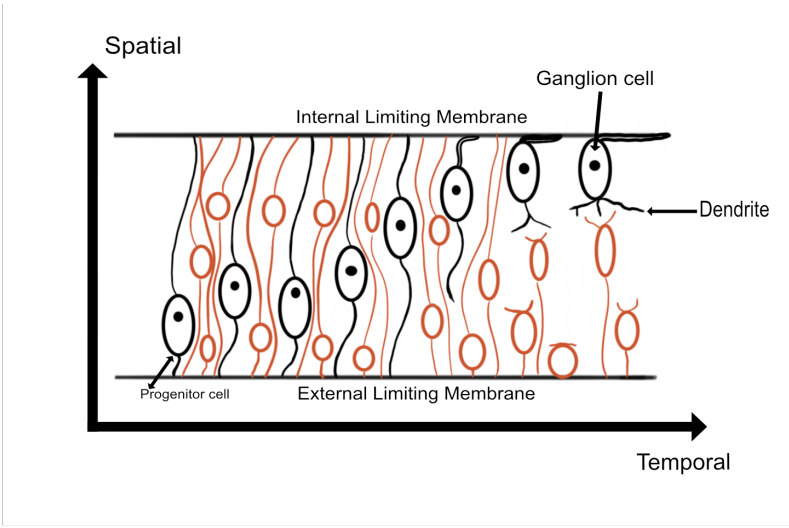


Figure 2. Spatial and temporal development of the retinal progenitor cell into a ganglion cell (black coloured) (By Deema Al-Hamdani).

The retinal ganglion cells have two extensions, one attached to the retinal outer limiting membrane and the other to the retinal inner limiting membrane on each side. The nucleus of the RGC develops near to the outer limiting membrane and then it develops a leading radial process that translocate the nucleus toward its future somal destination near to the inner limiting membrane in a process called nuclear translocation. During this process, the nucleus engages in an oscillating movement back and forth in the cytoplasm, called interkinetic migration (Sauer 1935).

The radial process grows and extends across the inner surface of the retina and gives rise to the developing ganglion cells axon, which happens even before the developing nucleus reaches its final destination.

The most cell-dense area in the developing retina is central and temporal to the optic nerve head, at the location of the future fovea. Ganglion cells in this region are highly packed but displacement occurs at the center leading to the formation of the foveal pit. Moreover, a naturally occurring programed cell death among the RGCs leads

to reduction in its numbers of up to the half (Mayordomo et al. 2003) (Chavarria et al. 2013).

Ganglion cell distribution continues radially from the center to the peripheral retina (Hu and Easter 1999) (McCabe et al. 1999) where ganglion cells are more densely distributed in the central retina compared to the periphery.

Ganglion cell density is highest around 17 weeks of gestation in the central retina and along the horizontal meridian. The number of RGCs are about (2.2-2.5) million between week 18-30 of gestation (Provis et al. 1985), later declining rapidly to an average of 1.2 (±0.2) million in adults (Jonas et al. 1990).

Formation of the Fovea

The fovea is the center of the macular area which is located temporal to the optic nerve and considered as the center of the field of vision and critical for high visual acuity and color vision. All types of cells in the fovea show specific changes that give the fovea its uniqueness. The outer nuclear layer (Gyllencreutz et al.) (Gyllencreutz et al.) which represents photoreceptors in the fovea, is composed of a single layer of only cones, compared to the peripheral retina in which this layer contains both cones and rods. As early as 20-27 fetal weeks there is a rod-free zone in the central fovea that measures about 1500-1800 μm (Xiao and Hendrickson 2000) (Hendrickson et al. 2008) but at birth it is about 1000 μm and at 45 months (about 4 years) of age it is about 650-700 μm due to centripetal migration and packing of cones (Yuodelis and Hendrickson 1986).

During the same fetal period (week 20-27) the ganglion cell Layer is much thicker than the periphery of the retina (Hendrickson et al. 2012). At week 25 of fetal age starts

the formation of the foveal pit by a central depression formed by outward displacement of the RGCs, the inner plexiform layers and the inner nuclear layer.

Failure of the formation of the optic pit leads to an underdeveloped fovea or foveal hypoplasia. The pit becomes larger in diameter and shallower until it takes its mature appearance at 15 months after birth. The process of widening of the pit continues up to 3.8 years after birth until there are no neurons occupying the pit's center. The morphology of the macula at this age is similar to a macula from a 13-year-old subject but the density of the cones continues to rise to almost double during this period (Hendrickson et al. 2012).

The developed macula is about 3 mm temporal to the optic disc with a diameter of about 5.5 mm (Figure 3), elliptical in shape and slightly elongated horizontally, and has these distinct regions:

1. Foveola: it is the center of the fovea. It averages about 350 μm in diameter. This area is consisting of cone photoreceptors only and it has the highest density of about 200 000 /mm^2. The center of the foveola is referred to as the umbo (100-200 μm across)
2. The fovea centralis: the central 1000-1500 μm in diameter, which involves both the foveola and surrounding area. The slope of the displaced inner retinal layers and the accumulated displaced inner retinal cells are within this area. This area is sometimes referred to as the clinical macula.
3. The Foveal Avascular zone (Montalban et al.): is a central area of about 500 μm in diameter and it is devoid of all vascular structures.
4. Parafoveal zone: It is the region around the fovea centralis, extending up to 2.5 mm around the center.
5. Perifoveal zone: Extends from the margin of the parafoveal zone to the edge of the macula (between 2.5-5.5 mm).

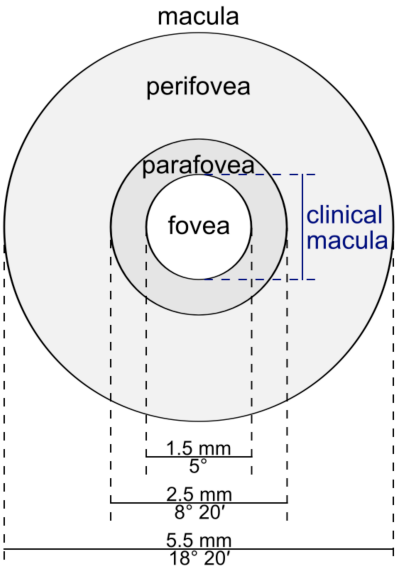


Figure 3. Macular regions and dimensions (reproduced from Wikipedia under a CC-BY-ND-4.0 license, creative commons)

Ganglion cell axons routing

The newly formed axon of the differentiated ganglion cell extends along the inner surface of the developing retina into the retinal nerve fiber layer or optic fiber layer and grows toward the optic disc (Hinds and Hinds 1974) (Zolessi et al. 2006).

As the axons grow, they are restricted to the inner surface of the retina on the vitreal surface by a balance between growth promoting factors and inhibitory guidance cues. In mice lacking these inhibitory factors, the RGC axons may continue to grow into the outer retinal layers (Thompson et al. 2006).

In the same manner there are many factors that act as inhibitory signals that prevent the axons from growing into the peripheral retina and other factors that act as promoting signals toward the optic disc (Thompson et al. 2006) (Kolpak et al. 2005). Once the axons reach the optic disc, they will be directed by the factors secreted by the glial cells surrounding the optic disc, for example netrin-1, to exit the eye, whereas

lacking netrin-1 factor leads to axonal miss-routing and optic nerve hypoplasia (Deiner et al. 1997). Several other factors play a role in this process of directing the axons to the optic disc (Birgbauer et al. 2000).) (Birgbauer et al. 2001) (Zelina et al. 2005).

After leaving the eye and entering the optic stalk, the growth and direction of the axons continues by the influence of several factors that may regulate whether the axons will continue to the same side or crossing to the other side of the brain and forming the optic chiasm, where some of these factors are expressed by the glia at the chiasm (Trousse et al. 2001) (Macdonald et al. 1997) (Nakagawa et al. 2000).

Considering the above data, it becomes clear that crossing at the chiasma to opposite side is an active process due to different repulsive, attractive, and permissive cues which help the RGC projections to determine laterality (Erskine and Herrera 2007).

1.1.2 Classification of the retinal ganglion cells

The retinal ganglion cell diversity makes the classification of these cells a complex process. As there is variation in anatomical localization in the retina, the ganglion cells may be divided as in the macula, outside macula, and peripheral retina. The ganglion cells may be divided based on their body size, density, shape, spread of dendrites, dendrite projection depth, size of the receptive field, or may be classified by electrophysiological characteristics or according to profiles of gene expression.

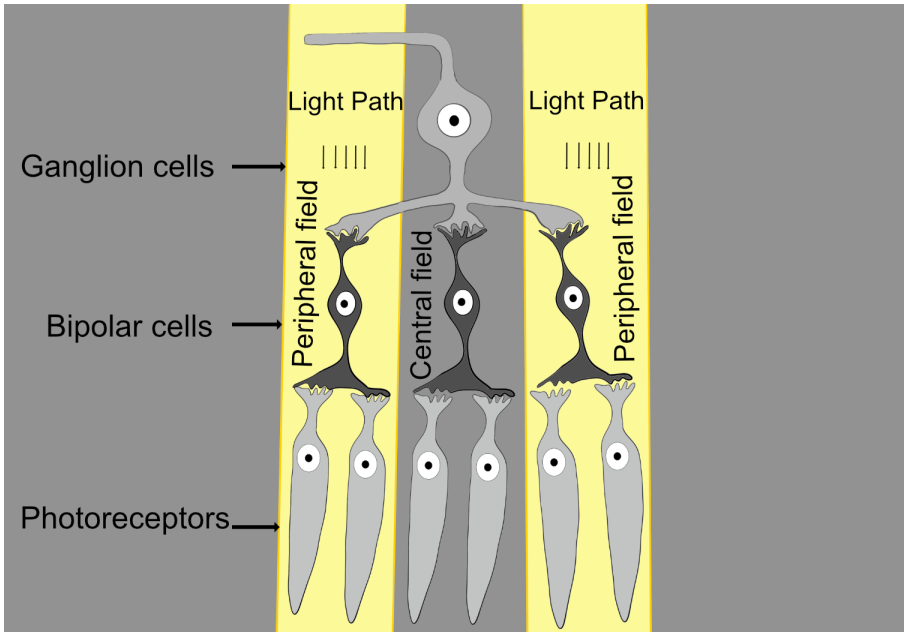
In general, in primates and human there are an estimated 18-20 different types of RGCs (D. Dacey 2004; Masri et al. 2019; Yamada et al. 2005). Lower mammals, however, have more RGC types. For example, there are over 40 RGC subtypes in the mouse despite the lower total number of RGCs (Rheaume et al. 2018).

It is worth noticing that similarities between RGCs in different species does not mean that these cells are identical, as differences in gene expression have occasionally been identified (Yan et al. 2020).

1.1.2.1 Functional classification

As the light enters the eye and reaches the retina, the photoreceptor cells are the responsible to capture and convert the light into an electrical activity. Humans have two major types of photoreceptors, the rods which are responsible for (black and white) vision and are more sensitive to light than cones by 25-1000 folds (Fain and Dowling 1973) (Tachibanaki et al. 2001). The cones are responsible for colour vision. There are three types of cones according to the colour they respond to, red, green, and blue. The signals from the photoreceptors will be passed to the bipolar cells, where another level of processing happens before it passes to the ganglion cells.

According to the response of the ganglion cell to stimuli it may be classified into an ON or OFF cell in which an ON-cell responds to a stimulus, as change in luminance, with excitatory action potential. An OFF-cell responds to the absence of stimulus and will be inhibited in the presence of light. In the central retina there is a 1:1 representation, i.e., one photoreceptor is connected via a bipolar cell to one ganglion cell and the ganglion cell is either an ON or OFF cell. Outside the macular area, the number of photoreceptors connected to a single ganglion cell is higher and may reach up to 1000:1 in the peripheral retina. In this case, and because of the of the high number of connections, the ganglion cell may respond differently according to which and how many photoreceptors are stimulated. This arrangement of photoreceptors connected to a single ganglion cell is called the ganglion cell receptive field (Figure 4 A).



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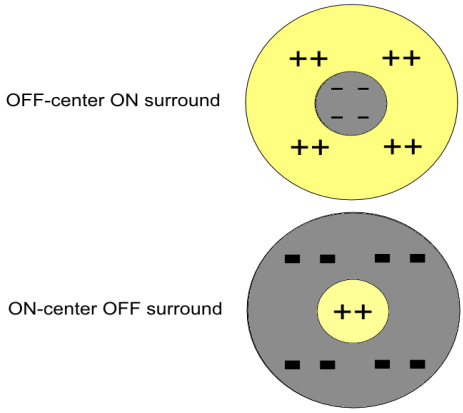

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Figure 4. A Schematic drawing of the ganglion receptive field. (A) The two central photoreceptors are connected to a bipolar cell which connects to the central dendrites of the ganglion cell and comprises the central field, while the four peripheral photoreceptors are connected to two different bipolar cells, and they connect to peripheral dendrites of the ganglion cell, comprising the peripheral receptive field. (B) If stimulating the peripheral field leads to activate the ganglion cell, then this cell is an OFF- center ON-surround cell (upper diagram) while if stimulating the center leads to stimulation of the cell then the cell is ON-center OFF-surround (lower diagram). (By Deema Al-Hamdani).

Some ganglion cells increase their electrical discharge rate if the center of its receptive field is stimulated and it will be suppressed when the periphery of its receptive field is stimulated; such a ganglion cell is called an ON-center OFF-surround ganglion cell and for simplicity is called an ON-center cells (van Wyk et al. 2009). If the ganglion cell is suppressed when the center of its receptive field is stimulated, it is an Off-center cell and will increase its electrical discharge rate when the periphery of its receptive field is stimulated (Figure 4 B). If different parts of the ON and OFF parts of the receptive field are stimulated, then the result of the excitation in the ganglion cell is the sum of the excitation of both areas (the center and the surround) (Werblin and Dowling 1969). Horizontal cells that are allocated between the photoreceptors and bipolar cells can modulate the response by both feedback and feedforward to both cells (Hirasawa and Kaneko 2003) and the same is true for amacrine cells allocated between the bipolar cells and the ganglion cells (Flores-Herr et al. 2001).

On the other hand, there is another type of ganglion cells called ON-OFF cells which are briefly triggered when the stimulus is turned on or off.

1.1.2.2 Morphological classification

RGCs can be classified according to the size of the cell bodies (somas); the ganglion cells with smallest cell bodies are the koniocellular (K) type which were discovered recently because of their minute size, and these project to the koniocellular layer of the Lateral geniculate body (LGB). The midget (Parvo) cell is a mid-size ganglion cell (D. M. Dacey 1993) and the largest is the parasol (Magno) cell type (Yamada et al. 1996).

Another morphological criterion upon which the cells can be classified is the type of dendrites, depth in the inner plexiform layer (IPL) and size of the dendritic field.

Some dendrites are spares in their distribution compared to others with normal distribution, others could be mono, bi- or diffusely stratified; dendrites may have normal shapes, thorny or recursive and may be spread across a small or larger field. The depth of the dendrites in the IPL and mode of stratification is of significant functional importance as grossly, dendrites located in the outer IPL contact the OFF bipolar cells and those in the inner IPL make synapses with the ON bipolar cells (D. Dacey 2004).

Another criterion for classification is the downstream connection or projections of the ganglion cells into the brain (discussed in detail in 1.1.4)

1.1.3 Types of retinal ganglion cells

According to the above criteria, there are different ganglion cell types which may have different functional, morphological, and anatomical characteristics. In humans there is some consensus on the major types of retinal ganglion cells such as the parasol, midget and koniocellular retinal ganglion and intrinsically photosensitive cells, while there is debate about other cell types which may comprise up to 40 different types depending on molecular criteria and retinal information streams (Reinhard and Münch 2021).

Midget (Parvo) Retinal ganglion cells (P-cells)

This type is the major RGC type in human retina and accounts for up to 70-80% of centrally located RGCs and about 50% of the peripherally located RGCs (Marshak 2009; Yan et al. 2020).

Midget cells have a small body with small field of dendrites centrally which become larger and with a wider field (up to 20 times wider) in the peripheral retina(U. S. Kim et al. 2021). These cells are also called the parvo or P-cell because it projects into the parvocellular pathway (D. M. Dacey 1993). These cells have a low contrast sensitivity but on the other hand they have a high chromatic opponency (Wiesel and Hubel 1966) and they are connected to long wave (L), red, or middle wave (M), green sensitive cones. Very few of these midget cells (OFF type) are connected to blue, short wave (S) cones according to recent studies (Tsukamoto and Omi 2015; Wool et al. 2019). Other studies done with electron microscopy showed that a small number of these cells might have a receptive field of the yellow-ON, blue-OFF type (Schiller 2010). These cells are also considered more sensitive to motion and flicker detection(Kelly 1981).

Approximately half of midget cells input comes from amacrine cells in the central retina while even more amacrine input comes for those cells in the peripheral retina and this input may play a role in the red-green opponency (Lebedev and Marshak 2007).

Parasol (Magno) retinal ganglion cells (M-cells)

This type is also considered as a major type of ganglion cell in primates' retina even if it is only comprising about 5% of all retinal ganglion cells in the central retina and about 15% in the periphery. The parasol RGCs project to the Magnocellular pathway to the LGN, hence the name M-cells (Ivan et al. 2023). M-cells have a larger cell body and receptive field than midget cells. Its dendrites stratify centrally in the IPL and the field of stratifications becomes wider with eccentricity. Its receptive field is also larger than the midget cells and receives input from the rod pathway as it is more peripherally located.

In contrary to midget cells, M-cells have a lower chromatic antagonism but higher sensitivity to luminance and contrast (Kaplan and Shapley 1986). M-cells additionally serve other functions such as motion perception, depth processing (Schiller 2010) and a response to transient changes in light stimulus.

Small Bistratified (K-cell) retinal ganglion cells

The smallest retinal ganglion cell that projects to the koniocellular pathway and it accounts for approximately 5-8% of all retinal ganglion cells (Masri et al. 2019). The K-cell dendrites stratify in both the inner and outer part of the IPL. K-cells are connected to the bipolar cells that exclusively receives input from the S-cones (blue) and are inhibited when light of long and medium wavelength is projected on them (D. M. Dacey and Lee 1994). For this reason its contribution to the yellow-blue colour vision is important (Szmajda et al. 2006).

Intrinsically photosensitive Melanopsin-containing ganglion cells (ipRGC)

This cell type is approximately 1% of all retinal ganglion cells and contains its own photosensitive pigment, melanopsin, which gives this cell its ability to respond directly to light even in the absence of connection to cones and rods (Munch and Kawasaki 2013).

These cells have a large cell body and a sparce but large dendritic field and abundance of mitochondria with higher concentrations of cytochrome-c oxidase (Ba-Ali and Lund-Andersen 2017). These cells are distributed mostly in the parafoveal area and at the far end of the nasal retina (La Morgia et al. 2010), and their function is to regulate the pupillary light reflex through projections to the midbrain and the circadian rhythms via the retinohypothalamic tract (Van Drunen and Eckel-Mahan 2021).

1.1.4 Connection to the Brain

Ganglion cell axons, and after leaving the eye via the optic disc, form the optic nerve. The optic nerve is divided into an intraocular segment or the optic disc (papilla); a retrobulbar segment after emerging from the bulb that is S-shaped to give mobility to the nerve during eye movements; an intracanalicular segment in which the optic nerve passes through the cranial bone from the orbit into the cranium; and finally, an intracranial segment where it enters the middle cranial fossa and onward until its nasal fibers crosses to the other side to form the optic chiasm. The myelin sheath is absent in the intraocular part and appears only after the axons leave the bulb through the lamina cribrosa.

Ibn Al-Haitham (965-1039 AC Basra-Iraq), known as Alhazen, considered as the father of the modern optics, described the anatomy of the optic pathway as detailed in

his book (Kitab al-Manazir) as he mentioned the retina, optic nerve, optic chiasm, and optic tract and gave them Arabic names as “Asab al-Basar” (Unal and Elcioglu 2009). But due to the lack of microscopes at that time one may notice they could not follow the fibers to their final destinations (Figure 5).

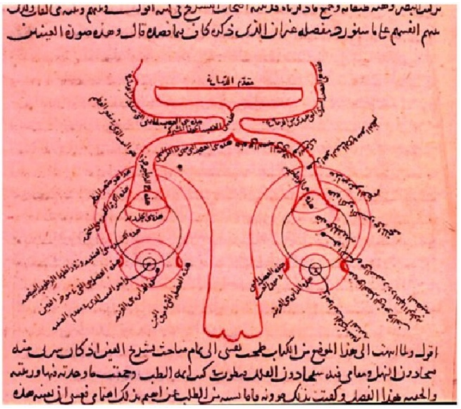


Figure 5. The structure of the human eye and optic nerve connections according to Ibn Al-Haytham, late 11th century. CE of copy of the Kitab al-Manazir (MS Fatih 3212, vol. 1, fol. 81b, Süleimaniye Mosque Library, Istanbul) (reproduced under a CC-BY-ND-4.0 license, creative commons).

The human retina has many different types of retinal ganglion cells which serve many different functions. And to add another level of complexity to the interpretation of visual function these cells project to different parts of the brain in which more specialized cell types receive and process the information. There are major routes for the projection of ganglion cell axons.

1.1.4.1 Retinogeniculostriate pathway (Visual pathway, or optic tract)

The ganglion cells' axons have an organized retinotopic map in the retina and along the visual pathway. Axons that emerge from the macular area continue to the temporal side of the optic disc and are called the maculopapular bundle. This serves the central part of the visual field and represents the central 5 degrees of the visual field. The nerve

fibers from above and below the macula (arcuate bundle) enter the optic disc just above and below the maculopapular bundle respectively (Fitzgibbon and Taylor 1996). More peripheral fibers enter the disc in a concentric manner, around the macula, and the most peripheral fibers enter at the superior and inferior poles of the disc. These fibers enter on the nasal side in an almost similar manner.

The fibers respect a horizontal line (horizontal raphe), i.e. a line (that divides the retina into superior and inferior halves and this line passes through the fovea (Ballantyne 1947).

Another significant observation is that more central fibers enter the optic disc deeper to the more peripheral fibers. Within the optic nerve, these fibers generally maintain their spatial representation until they reach to the optic chiasma where more nasal fibers (nasal to an imaginary line passing through the center of the fovea) will cross to the other side (Harrington and Drake 1990) , decussate to form the optic chiasm, and join the temporal fibers of the other optic nerve to form the optic tract after the chiasm (Hoyt and Luis 1962; Sarlls and Pierpaoli 2009). About 53% of all retinal ganglion cell axons decussate and the remaining 47% continue to the same side (BCSC 2021).

At the lateral geniculate nucleus, the axons of the ganglion cells arrive at distinct cell layers as it has six layers and three distinct cell types: Parvocellular, Magnocellular, and Koniocellular. The two ventral layers are M layers while the dorsal four layers are P layers; the K layers are spread between those layers.

Axons of the cells from the same side ends at and synapsing to cells at layers 2,3 and 5 while decussated axons from the other side synapsis at layers 1,4 and 6 (Callaway 2005). After making the synapse at the LGN the second order neuron passes and makes the optic radiation and via the internal capsule reaches to the visual cortex (V1 area) at the occipital lobe (Figure 6).

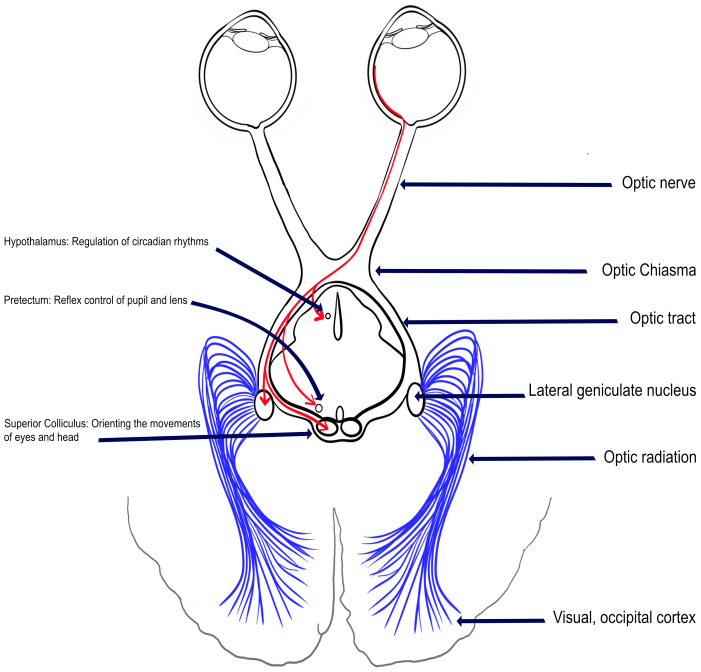


Figure 6. Visual pathway and different connections of the retinal ganglion cells inside the brain. (By Deema Al-Hamdani).

1.1.4.2 Connections to the brain stem (Accessory optic system)

A special group of ganglion cells which are direction-selective, connect to the mid brain at:

a. Nucleus of optic tract and dorsal terminal nucleus (NOT/DTN)

b. Medial and Lateral Terminal nuclei (MTN & LTN)

The former is responsible for horizontal image stabilization while the latter for vertical image stabilization (retinal slip) (Borst and Euler 2011; Simpson 1984).

1.1.4.3 Intrinsically photosensitive retinal ganglion cell routes

The ipRGC responds to light stimuli independent of the cone and rod stimuli because of the presence of the photosensitive pigment Melanopsin (Hattar et al. 2002). These cells do not connect to the visual cortex and hence are considered to control non-image forming behaviors (Dhande and Huberman 2014). These cells connect the retina to two major locations in the brain serving two distinct functions:

A. Hypothalamus (Supra-Chiasmatic nucleus, SCN) which is considered as the circadian clock.

B. Midbrain (Olivary pretectal nucleus, OPN): The axons of the ganglion cells pass without any synapse at the LGN and continue to the OPN on both sides and from there another neuron projects to the Edinger-Westfall Nucleus (EWN) to control both pupils via the parasympathetic pathway which travels with the oculomotor nerve.

Because of these connections, it is hypothesized that the ipRGC routs serve to connect the light-dark cycle to the endogenous circadian rhythm (Guler et al. 2008).

Until recently it was proposed that ipRGC that connect SCN and OPN were of the same type, but later studies in the cat showed that the circadian function and pupillary light reflexes are mediated by distinct ipRGC populations (Chen et al. 2011). Other studies showed that there are at least 5 different ipRGCs subtypes which project to many different central targets (Guler et al. 2008).

1.1.5 Mechanisms of RGC injury

The ganglion cells as other cells in the body are vulnerable to different processes that may lead to cell injury, damage or even cell death. Because of its unique anatomical position, with cell bodies in the retina and axons extending outside the bulb into the orbital and then the cranial cavities, these cells may be affected by pathological processes inside the eye or the brain or in the orbit.

Direct trauma (Traumatic optic neuropathy) caused by either penetrating injury or skull fracture with a bone piece traumatizing the optic nerve and axonal damage. This damage will lead to leakage of intracellular contents and initiation of different processes like formation of free radicals and even excitotoxic effect of glutamate, which is the neurotransmitter in the ganglion cell, disruption of axoplasmic transport, activation of microglia as well as proliferation of macroglia and disruption of the blood brain barrier and finally death of the retinal ganglion cell. Retrograde cell death after axonal injury is clear.

On the other hand, in cases of indirect traumatic optic nerve injury as in cases of non-penetrating skull trauma there might be a direct damage to the axons or damage to the vascular supply to the nerve (Anderson et al. 1982) (Kumaran et al. 2015) by different mechanisms such as hematoma or oedema of the optic nerve sheath (Kline et al. 1984), tearing of the sheath (Gross et al. 1981), or tugging of the optic nerve from the brain at the optic canal (Smith DH 2000).

Raised intra cranial pressure leads to swelling of the optic nerve head and dysfunction of the ganglion cells to a variable extent. Cerebrospinal fluid is produced mainly by the choroidal plexus in the lateral ventricle (Donaldson 1979) and it circulates in the brain ventricles to later reach the subarachnoid space (Eklund et al.

2007). The subarachnoid cavity continues around the optic nerve and ends in a cul-de-sac configuration in the retrobulbar region.

The ganglion cell body and its terminal synapses maintain an intra cellular communication path via the cellular plasma, called axoplasmic transport or flow. There are two types of axoplasmic flow, antegrade where the direction of movement of macromolecules and organelles is from the cell body to the synaptic terminal, while the flow in the other direction toward the cell body is called retrograde axoplasmic flow. This flow is vital for the ganglion cell and any interruption of the flow leads to stagnation of the axonal fluid and swelling of the fibers.

In cases of increased intracranial pressure, pressure rises around the optic nerve and compresses its fibers which leads to disturbance in the axoplasmic flow, accumulation of the fluid in the prelaminar region and swelling of the fibers (Wirtschafter et al. 1975). Inside the eye this can be seen as swelling of the optic disc, haziness of the RNFL, and in severe cases even swelling of the adjacent retina. In more severe cases even stagnation of the blood flow in the small venules may lead to small hemorrhages. The underlying mechanism of papilledema due to raised intracranial pressure is supposed to primarily be a mechanical and nonvascular phenomenon (Wirtschafter et al. 1975). On the other hand, even if the vascular theory is not as strongly supported as the mechanical theory, there is compelling evidence that supports it (Hayreh 2016; Trobe 2011).

Hypoxic-ischemic injury. The retina is metabolically highly active (Cohen et al. 1965) and is among the tissues with high oxygen demands, even more than the brain (Ames 1992). The inner retinal layers including the RGC and RNFL are very sensitive to hypoxia (Janaky et al. 2007).

Among the diseases that may lead to retinal hypoxia and damage to the RGCs is DM (Diabetes Mellitus), systemic vascular diseases such as cardiovascular disease, and ischemic ocular syndrome.

The hypoxia may through different cellular mechanisms lead to cell death as induction of expression of Hypoxia Inducible Factor 1 Alpha (HIF-1α) (Bernaudin et al. 2002), and nitric oxide synthase which leads to increased specific subtypes of nitric oxide which is toxic to retinal ganglion cells (Neufeld et al. 2002). Hypoxia leads to excess glutamate production which becomes excitotoxic to RGCs and other neurons (Benveniste et al. 1984). Hypoxia may also lead to the release of free radicals, increased intracellular calcium and increased expression of vascular endothelial growth factor (VEGF). All these mechanisms lead to cell damage and death because of the hypoxic ischemic insult.

Inherited neuropathies lead to an irreversible visual loss due to RGC loss. The most common types of hereditary neuropathies are dominant optic atrophy (DOA) and Leber Hereditary optic neuropathy (LHON). In both cases the defective genes are responsible for mitochondrial dysfunction which affects vital processes like oxidative phosphorylation (Carelli and Chan 2014) and complex-1 mitochondrial dysfunction (Stenton et al. 2021). As a result, there will be disturbance in energy production, glutamate accumulation and increased production of reactive oxygen species (ROS) (Maresca and Carelli 2021). The smaller ganglion cells with smaller mitochondrial reserves are the most vulnerable and these are predominantly located in the papillomacular bundle (Sadun et al. 2000) while ipRGCs have been preserved in LHON.

Degenerative mechanism. One of the most common examples of RGC degenerative diseases is glaucoma. Glaucoma is a chronic eye disease and one of the leading causes of irreversible blindness in the world's elderly population. It is still unclear what causes glaucoma, but the intra-ocular pressure (IOP) plays a key role. And it is unclear if the IOP initiates the degenerative process, as some subjects have no sign of glaucoma despite high IOP as in ocular hypertension meanwhile some studies show that elevated IOP induces cell stress and lead to retinal astrocyte activation and hypertrophy (Macanian 2022). Mechanisms by which the IOP may cause cell death are unclear but some postulate that elevated IOP causes a mechanical pressure on the nerve fiber layer against the ridged lamina cribrosa or at Elschnig´s ring causing disturbance in axoplasmic transport of neurotrophic factors (Johnson et al. 2000). While the ischemic theory states that hemodynamical alterations at the optic nerve head may act independently and lead to optic nerve head atrophy (Flammer 1994) Other claimed mechanisms include upregulated autophagy (Deng et al. 2013), age-dependent accumulation of amyloid-Beta proteins (Aβ) in RGCs (Guo et al. 2007).

RGC death in glaucoma occurs through apoptosis (Garcia-Valenzuela et al. 1995) and the most vulnerable RGC type to be damaged is the larger cells in the magnocellular pathway (Glovinsky et al. 1993; Quigley 1995) possibly due to calcium permeable receptors or differing metabolic requirements of these large cells (Wang et al. 2020).

Inflammatory-demyelination process. As a classic example of inflammatory-demyelinating disease that affects ganglion cells and its axons is Multiple sclerosis (MS), which is associated in about 70% of cases with optic neuritis during the disease course (Bando 2019). The ultimate cause of optic nerve damage in MS is demyelination and axonal damage which is mediated by inflammatory cells as activated autoreactive

and myelin-specific T cells, B cells, macrophages and dendritic cells which release cytokines and other proinflammatory mediators (Ciapă et al. 2022) resulting in oligodendroglial cell death-mediated demyelination. There is some evidence that inflammation of the retinal vascular endothelium resulting in perivascular cuffing as well as optic nerve sheath oedema may precede the demyelination and leads to the myelin breakdown (Lightman et al. 1987). Demyelination and persistence of pro-inflammatory processes leads to axonal loss in the parvo and magnocellular pathways (Cao et al. 2011). In demyelinating optic neuritis acute stage, the blue-yellow defects are more common while the red green changes become more prominent in chronic stages (Katz 1995).

1.2 Optical Coherence Tomography

1.2.1 Principles and basics

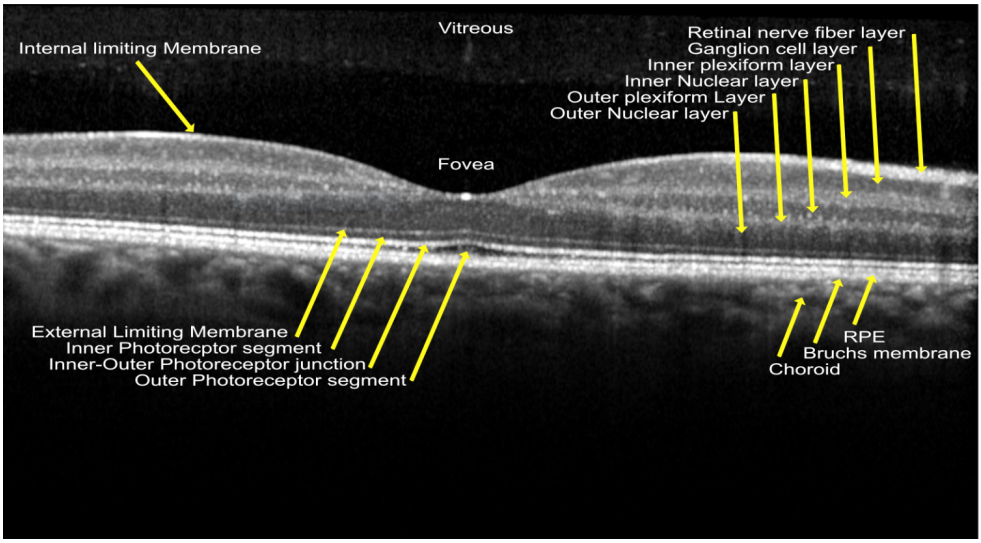
Optical Coherence Tomography (OCT) refers to a non-invasive imaging technique which uses low coherent light interferometry. Interferometry generally uses two light beams from one light source and extracts information from their interference (Hecht 2017).

In the case of OCT, the device uses low-coherent white light which is split by a beam splitter into two beams. One of the beams is reflected from a mirror which is called the reference beam or arm, and the other beam is directed to the tissue to be investigated and is called the sample beam or arm (D. Huang et al. 1991b) and its reflectance/scattering depends on the tissue. Both reflected beams are then combined by the splitter and the resulting interference fringe or fringes will be directed to a photodetector. The signal is then processed through a computer-based system to analyze and present the results visually. By calculating the delay in time or phase

difference between the two beams, the OCT device converts the information into depth data. Every calculated beam gives a reading called an A-scan, and by combining different A-scans the devices can provide a 2-D picture of the tissue called a B-scan and referred to as an optical biopsy (Fujimoto et al. 1995) (Figure 7).

1.2.2 Types of OCT

Several types of OCT devices were produced during the last 30 years and the technology of these devices has evolved rapidly. The earlier devices were slower with lower axial resolution and with very limited ability for segmentation were primarily used early for imaging the anterior segment (Izatt et al. 1994).



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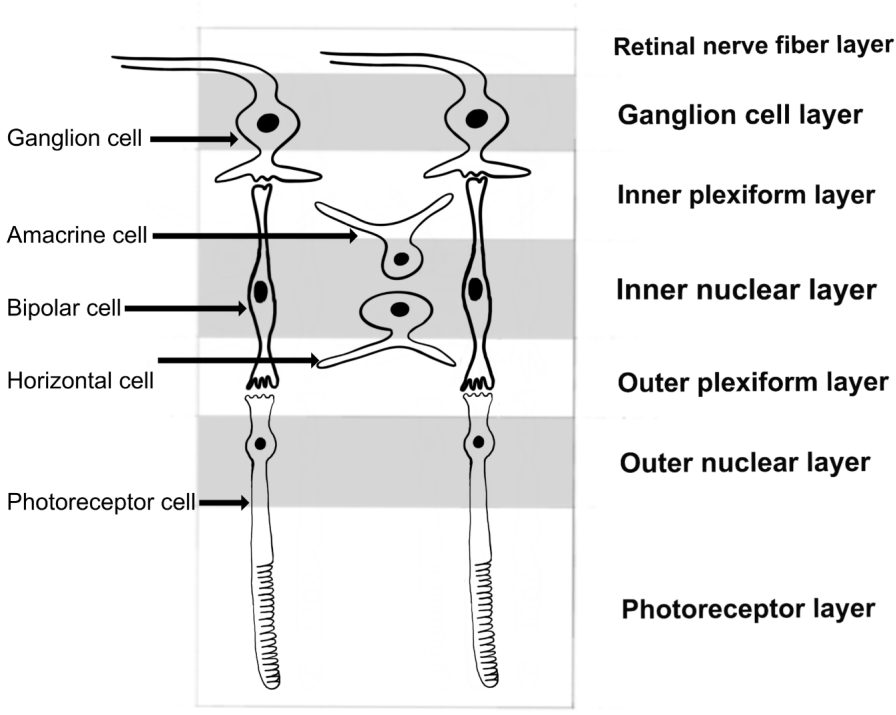

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Figure 7 A. OCT B-scan image shows different retinal layers (Image by Abbas Al-Hawasi). B. a diagram shows retinal cell locations in different retinal layers (By Deema Al-Hamdani).

1. Time-Domain OCT (TD-OCT)

This is the oldest generation of OCT which uses a moving mirror as a reference arm. This mechanical movement limits the device to 400 A-scans/second and an axial resolution of 10 μm. These first devices used an 800-nm low coherence laser diode (D. Huang et al. 1991a).

2. Spectral-Domain OCT (SD-OCT)

This is also known as Fourier-Domain OCT. It uses a light source with broader bandwidth, to simultaneously send multiple wavelengths of light into the eye. A spectrometer at the output then separates the spectrum of light returned from the eye, with each wavelength carrying different interference information from arms of fixed length, thus requiring no moving mirror. All wavelengths are received at the same time, which is much faster than the earlier time-domain method. In the spectral domain it is possible to take up to 40 000 A-scans/second which makes it not only faster but more accurate with higher axial resolution (up to 2.1 μm) (Wojtkowski et al. 2004). The later versions of this type of OCT can take up to 70 000 A-scans/second.

Two types of SD-OCT were used in this project, more details will be further described in the methods below.

3. Swept-Source OCT (SS-OCT)

In this type of OCT there is no spectrometer, and the laser diode is replaced by a tunable laser as a light source (Lavinsky and Lavinsky 2016). With up to 100 000

A-scans/second it is much faster and can take wide scans involving both macula and optic disc in one session (12x12 mm wide scans)(de Carlo et al. 2015). Some prototypes of SS-OCT can take up to 400 000 A-scans/second (Potsaid et al. 2010).

4. OCT Angiography (OCT-A)

This type of OCT is more directed to detect motion in tissue caused by blood flow and is used to image the microvasculature of the retina and choroid (Koustenis et al. 2017). It uses either spectral domain or swept source OCT principles. When the reference and reflected lights recombine, they create an interference pattern. The phase changes in interference pattern are caused by the movement of blood cells resulting in variations in the intensity of the interface pattern. These phase changes along with the variation in intensity will be detected by a sensor in the OCT-A device. These variations will be considered as blood movement in vessels and a corresponding image will be generated. The device can produce both en-face (single plane) and 3D pictures(Spaide et al. 2015).

5. Adaptive optics OCT (AO-OCT)

These OCT devices are used in combination with advanced adaptive optics to correct for optical aberrations in the eye, to achieve extremely high resolution that allow for cellular structural visualizations (Pircher and Zawadzki 2017). The adaptive optics technology is adapted from astronomical photographing technologies which was used for the imaging of distant stars.

6. Confocal Scanning Laser (line-field) OCT

Other terms that are used are confocal scanning laser ophthalmoscope OCT (cSLO-OCT). In ordinary OCT devices the focus on the examined tissue is achieved by optical solutions with limited ability to exclude optical aberrations from the eye. The cSLO-OCT is provided with a high numerical aperture microscope objective to image with high lateral resolution (Webb et al. 1987). The small aperture provides a confocal gate which prevents most of the scattered light from reaching the sensor and increases the sharpness of the image.

Furthermore, new OCT technologies allow not only for imaging tissues but for using different technologies to get higher resolution (submicron) measurements, quantitative retinal blood flow measurement, spectral imaging with oximetry and measurement of mechanical tissue properties (Aumann et al. 2019).

1.2.3 Advantages and disadvantages of OCT examination

In daily practice, capturing OCT images and operating the device is easy with minimal need for training or experience.

- The examination is rapid without waiting lists when the patient is at the clinic.
- Non-contact, in most cases there is no need for pupil dilatation, and without any discomfort for the patient.
- The examination is harmless as it does not use ionizing radiation as in cases of CT-scans.
- Minimal cooperation from the patient is required.

- The produced image is detailed at the micrometer level and provides 2D and 3D configurations with details about the thickness, geometry as well as vascular structure.
- The ability for follow up imaging at the same tissue points.
- The ability for segmentation of different tissue layers.
- It is a very cost-effective investigation method as the device needs minimum service.
- There are many variants as desktop models, with minimal requirement for space; those are suitable for outpatient examinations. Portable models are suitable for unconscious or in-bed patients. Many models nowadays are incorporated within operation microscopes or even in table-based slit lamps.

On the other hand, there are some disadvantages with OCT as:

Many different manufacturers with different standards and methods of examination make the examinations by different devices not comparable.

The measurement may be affected by ocular pathologies as opacities in the media (van Velthoven et al. 2006), optical imperfections as refraction errors (Luo et al. 2006) and length of the bulb (Takeyama et al. 2014).

2. Aim of the thesis and research question

As the ganglion cells embryological origin is similar to the brain and as it keeps connection with different parts of the brain, there is a strong anatomical and physiological connection between the intraocular ganglion cells and its axons with the brain. Ocular examination with optical coherence tomography is a non-contact, comfortable, risk-free and, even in the hands of the minimal experienced operator, is a very fast and cost-effective method of examination.

The main research question of the thesis is to investigate whether the examination of the retinal ganglion cell and its axons at the retinal nerve fiber layer by optical coherence tomography can reflect different physiological and pathological changes in the eye and the brain.

To address different physiological and pathological mechanisms at different levels, we needed to choose different subject groups including both healthy subjects and those with diseases, and to examine them with different OCT devices.

Within this context, to answer the main question we planned to divide it into four sub questions to be answered by four different studies.

2.1 Optic neuritis affecting retinal ganglion cells and its axons (RNFL). Research question 1: How do serial OCT examinations reflect pathological changes in RNFL and GCL-IPL in patients with optic neuritis? Paper I.

Inflammation of the optic nerve could be isolated or could present as a part of more systemic disease such as multiple sclerosis, and it may affect the intra-ocular part and (called papillitis) or may affect the retrobulbar part and is called (retrobulbar optic

neuritis). The axons of the ganglion cells are affected primarily in all cases of acute optic neuritis.

For this purpose, the first study is designed to investigate changes in retinal nerve fiber layer at the optic disc and ganglion cell layer in the macular area in patients with acute optic neuritis.

2.2 Effect of elevated intra-cranial pressure on optic nerve head and macular parameters

Research question 2: How does OCT measurement of the retinal nerve fiber layer and macular ganglion cell layer reflect intra cranial pressure changes? Paper II.

The second aim was to investigate and identify reliable and sensitive measurements of the optic disc and macula by measuring RNFL and GCL-IPL in a group of patients with elevated intra-cranial pressure without any other neurological diseases; as in patients with Idiopathic Intra-cranial Hypertension (IIH). We decided to measure not only the retinal nerve fiber layer around the optic disc but to take into consideration other optic nerve head parameters as it is composed of the axons at the disc.

2.3 Physiological factors effect on retinal ganglion cell thickness

Research question 3: How do different physiological factors, changes, affects the thickness of the retinal ganglion cell layer? Paper III

This study's aim was to identify how different factors affect the GCL thickness and if it may reflect similar brain changes. Different physiological factors affect the eye and

brain, but is it possible with an OCT examination of the retinal ganglion cells at the macula to estimate and identify these changes?

2.4 OCT measurement of RNFL and GCL-IPL as a biomarker for disease course

Research question 4: Can longitudinal measurement of RNFL and GCL-IPL identify benign course of a disease? Paper IV.

Here the aim was to investigate the longitudinal changes in peripapillary retinal nerve fiber layer and the macular GCL-IPL layer in a unique group of patients with Multiple sclerosis who have a benign disease course compared to other MS patients.

3. Materials and Methods

3.1 Population

In Paper I, the population consisted of a group of 4 female patients presenting to the ophthalmology department, Linköping University Hospital, Sweden with signs and symptoms of optic neuritis. Patients, after thorough ophthalmological examinations, were referred to the neurology department of the same hospital for further investigation and follow up.

In Paper II, the study population consisted of a group of 7 patients with already diagnosed Idiopathic Intra-cranial Hypertension (IIH) and their follow up visits at the neurology department, Linköping University Hospital, Sweden. All patients were female with Body mass Index more than 25kg/m^2. Five of them were on therapy with acetazolamide and occasional tapping with lumber puncture. Another healthy group of 18 age- and sex- matched subjects was enrolled as a control group.

In Paper III, 60 healthy subjects between 12-76 years of age, recruited from visitors to the ophthalmology department, Linköping University Hospital, Sweden. They have been examined between October 2015 and January 2019. Male to female ratio was (27/33); 55% of the population were female. Exclusion criteria were any ocular diseases other than mild cataract, history of any ocular trauma, any history of cardiovascular diseases, Diabetes Mellitus, history of cerebrovascular accident (CVA) or carotid artery disease, any neurological disease or history of neurological disease in the family such as Multiple Sclerosis, Alzheimer's or Parkinson's disease, history of neurotoxic medication such as ethambutol or radiotherapy to the head-neck region.

In Paper IV, the population consisted of a group of 20 patients with Benign Multiple Sclerosis (BMS) with follow up at the neurology department, Linköping

University Hospital, Sweden; and 26 healthy controls. Inclusion criteria for BMS patients was based on clinical definition defined by an Expanded Disability Status Scale (EDSS) (Kurtzke 1983) scores of ≤ 2 at 10 years after the first signs of MS or EDSS score of ≤ 3 after 15 years with no treatment or first line treatment (interferon, Copaxone).

The exclusion criteria were any ophthalmological disease that may affect the OCT (Optical Coherence Tomography) measurements such as glaucoma, macular degeneration, or Diabetes Mellitus. The study was conducted between March 2013 and April 2019.

3.2 Clinical examinations

Paper I was designed to evaluate the longitudinal peripapillary retinal nerve fiber layer thickness (RNFL) and macular ganglion cell layer - inner plexiform layer (GCL-IPL) thickness changes in patients with signs and symptoms of unilateral acute optic neuritis but with no previous history of neurological diseases. Ophthalmological evaluation included visual acuity test (VA) using Snellen's chart, color vision test with Hardy Rand and Rittler plates (HRR), visual field (VF) examination with Humphry perimetry, and visual evoked potential (VEP) using the checkerboard pattern reversal stimulation. OCT examination was done serially on separate occasions, the first one 2-3 weeks after optic neuritis onset (OCT examination and details given below).

In Paper II The IIH group underwent both ophthalmological and neurological examination including fundus photography and visual field examination, Lumbar puncture (LP) with measuring of the opening pressure in cm H_2O, Cerebrospinal Fluid (CSF) analysis, Magnetic Resonance Imaging and MR venography or Computerized

Tomography venography. OCT examination was done a half hour before LP with CSF removal in all cases and in two cases serial OCT examinations were done on 3-4 occasions, 1-3 months after CSF tapping.

In Paper III all included subjects were examined clinically and by visual acuity with Snellen's chart, autorefractometry and keratometry for refraction errors by Topcon® autorefractor-keratometer (Topcon Corp., Japan) or reading of actual eyeglasses, and Axial length measuring by Carl Zeiss® IOL (intraocular lens) master 700 (Carl Zeiss, Meditec AG, Germany).

In Paper IV all BMS patients were already examined with MRI and LP for the routine CSF test. All cases were examined ophthalmologically and by OCT at least on two occasions, with the first occasion at enrollment, considered as the baseline visit.

3.3 Optical Coherence Tomography examinations

3.3.1 Devices used in the project

Two devices had been used in all 4 Papers to examine the subjects. The Cirrus HD-OCT (model 4000, Carl Zeiss Meditech, software version 6.5) was located at the neurology department and was used to examine subjects in Papers I, II and IV. This device, according to the manufacturer, is a SD-OCT (Spectral Domain Optical Coherence Tomography) which uses an 840 nm superluminescent diode (SLD) which takes 27 000 A-scans/second with a scan depth of 2 mm. Its axial resolution is 5 μm and the transverse resolution is 15 μm (Cirrus HD-OCT 4.0 User Manual, specifications 10-1).

The second OCT device which is located at the ophthalmology department is a Heidelberg SPECTRALIS OCT system HRT II (Heidelberg Engineering, Germany).

This device was used in Paper III and is also a SD-OCT with infra-red cSLO technology and TruTack™ Active eye tracking system. It uses an 870 nm SLD, and is capable of 40 000 A-scans/second with scan depth of 1.9 mm and axial resolution of 3.9 μm and 14 μm of transverse resolution (SPECTRALIS® HRA+OCT manual 2012).

3.3.2 Examination protocols

For measuring the RNFL around the optic disc we used the glaucoma RNFL program, optic nerve head (ONH) with active anatomical positioning system (APS), at 3.5 mm ring around the optic disc center, in the Heidelberg Spectralis in Paper III. In Papers I, II and IV, the Cirrus HD-OCT was used with the built in Optic disc cube 200x200 centered on the optic nerve head with the thickness ring of 3.46 mm in diameter around the optic nerve head center.

For ganglion cell thickness and volume measurement we used the Macular cube 512 x 128 centered on the macula with 6 x 6 mm with the Cirrus HD-OCT in Papers I, II and IV. In Paper III we used the Retina dens 30 x 20 degrees built-in program of the Heidelberg Spectralis OCT. The thickness map was calculated on a 6.1 mm circle with resolution of 512 x 496.

In all macular measurements we used the built-in software of the device for segmentation except in Paper III where we manually corrected any segmentation errors as centration of the map ring or segmentation of the GCL. All macular data were according to the 6 mm ring according to the ETDRS (Early Treatment Diabetic Retinopathy Study) quadrants. In Paper III we further mathematically calculated

central 1 mm, mean inner 3 mm, and mean outer 6 mm rings as well as mean superior, inferior, nasal, and temporal sectors as in the Figure 8.

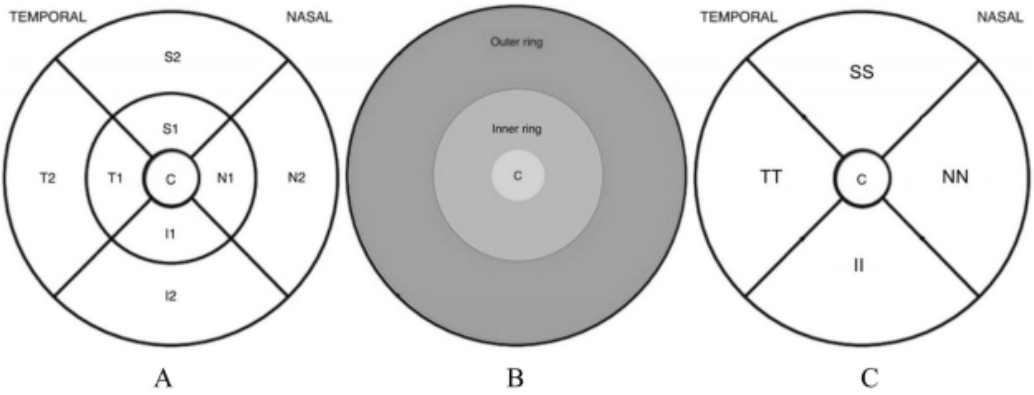
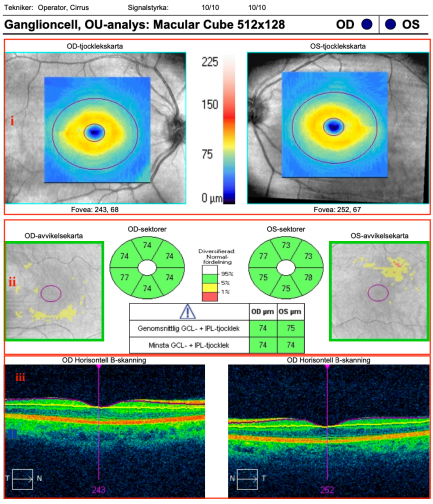


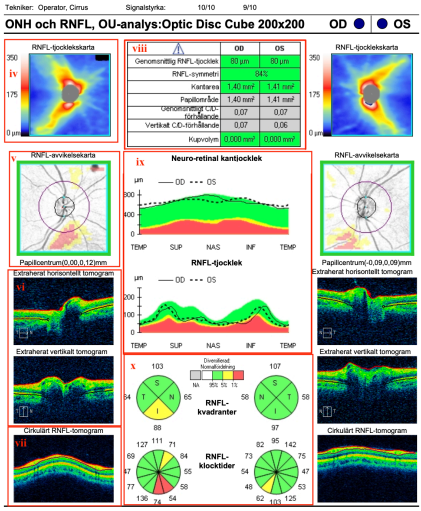
Figure 8. Division of retinal measurements into different anatomic sectors: (A) the 6 mm ETDRS quadrants sectored by the OCT device. S (superior), T (temporal), N (nasal) and I (inferior), 1 for inner sector and 2 for outer sector. (B) Dividing the measured area into central 1 mm ring- C, inner ring 3 mm diameter and outer ring 6 mm. (C) Dividing the inner and outer ring into SS (mean superior of S1 and S2 in figure-A) TT (mean temporal, T1 and T2 in figure-A) NN (mean nasal, N1 and N2 in figure-A) II (mean inferior, I1 and I2 in figure-A) From Paper III, reproduced under a CC-BY-ND-4.0 license, creative commons.

3.3.3 Optical Coherence Tomography reports and data

The built-in software for both OCT devices were used to generate thickness reports of the RNFL and macular GCL (Ganglion Cell Layer) in the Heidelberg device and GCL-IPL in the Cirrus HD-OCT, as shown in Figure 9 A and B from Paper IV. Panels C and D were taken from data included in Paper III.



(**A**)



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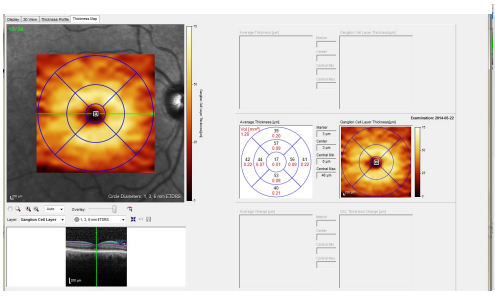
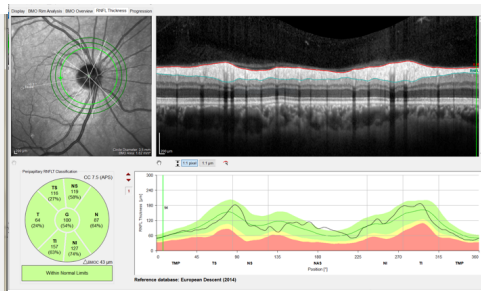


(**C**)



(**D**)

Figure 9. The OCT B-scans of the (A) macular GCL-IPL and (B) OCT of optic disc of a healthy subject by the Cirrus HD-OCT: (i) color coded GCL-IPL layer thickness, (ii) Thickness deviation map with macular data, (iii) B-scan and segmentations, (viii) Data table of the peripapillary RNFL, (v) RNFL thickness deviation map from normal thickness, (vi) horizontal and vertical RNFL thickness tomogram, (vii) circular RNFL thickness tomogram, (viii) data table with average RNFL thickness and optic disc parameters, (ix) neuro-retinal and RNFL thickness graph with normative data, and (x) thickness graph in quadrants and clock hour. (C) Ganglion cell layer thickness and (D) the peripapillary nerve fiber layer thickness by the Heidelberg Spectralis OCT. A&B From Paper IV, reproduced under a CC-BY-ND-4.0 license, creative commons.

4. Results

4.1 Paper I

Clinical outcomes

All 4 cases were proven to have MS (Multiple Sclerosis) later during the follow-up visits as determined by MRI and CSF analysis. Impaired visual functions such as visual acuity and visual field were normalized except for mild color vision impairment. All patients showed prolonged P100 latency in the eye with optic neuritis.

OCT (Optical Coherence Tomography) data

At the first OCT examination all cases showed an increase in the peripapillary RNFL thickness in the eye with optic neuritis. After 3 months there was a reduction in RNFL thickness, but it was still thicker than the contralateral eye without optic neuritis until after 6 months, where the thickness reduced to below that of the contralateral eye except for 1 case with intrabulbar optic neuritis where the patient had a thicker RNFL up to 9 months after the onset (Figure 10).

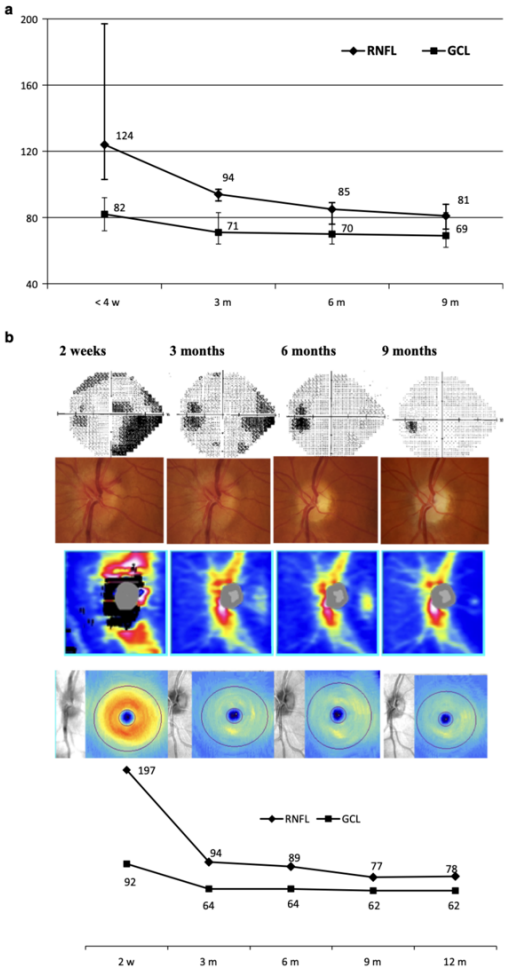


Figure 10 (a) Changes of retinal nerve fiber layer (RNFL) and macular ganglion cell layer (GCL) thickness (micrometer) from four consecutive patients over 9 months after acute ON onset. A similar trend was observed in all four patients: RNFL thickness decreased over 9 months, while GCL thickness became stable already 3 months after ON onset. w weeks, m months. (b) A 49-year-old woman (case 1) with acute intra bulbar ON in left eye. Visual acuity was 0.8 with moderate visual field defect that improved to 0.9 with normal visual field within 9 months after ON (panel 1). Initial fundus examination showed optic disc swelling which vanished leaving slight optic disc atrophy (panel 2) within 6 months. Mild color vision defect remained. RNFL was initially thicker but successively reduced over 9 months to a constant value (panel 3 and curves). GCL in macula was thicker in ON- than in non-ON-eye at week 2, but became abnormally thin within 3 months, followed by a constant value over a 9-month period (panel 4 and curves). The patient had relapse 6 months after ON onset, during interferon-beta therapy. For 7 months after ON onset, she has had received on-going natalizumab therapy. From Paper I, reproduced under a CC-BY-ND-4.0 license, creative commons.

4.2 Paper II

Clinical outcomes

All 7 female patients were on weight reduction therapy and therapy for reducing the intra cranial pressure except for two cases who were in remission and had no treatment. Five of the patients had clinical papilledema. No ocular disease was found except for cataract in one case. The healthy control group (n=18) was between 25-51 years of age (37±8) without history or symptoms of neurological diseases. Of the two cases which were followed longitudinally, the first one had severe persistent headache and visual symptoms, but the ophthalmological examination revealed normal visual acuity, color vision and there were no visual field defects, while fundoscopy showed moderate to severe papilledema, more on the left side. The second case had recurrent migraines and visual symptoms exacerbated by effort. Early during ophthalmological examinations, the patient had a slightly hyperemic optic disc but with normal vision and visual fields.

OCT data of the optic nerve head, RNFL and GCL-IPL

All IIH (Idiopathic Intra cranial Hypertension) cases had a RNFL and GCL-IPL thickness within the normal ranges of the healthy population ($P>0.05$), On the other hand, they had thicker neuro-retinal rim and larger rim area as well as lower cup-to-disc ratio ($P<0.05$). All cases except for one eye in cases 5 and 7, had zero cup volume, while the cup volume in the remaining two eyes was extremely low ($P<0.05$). The only optic nerve head parameter which was not significantly different between the groups was the disc area ($P>0.05$).

The two cases which were followed longitudinally after repetitive CSF removal showed no significant change in rim thickness, cup volume or cup-to-disc ratio

irrespective of CSF-ICP change or the removed CSF volume. However, the changes in RNFL thickness took 1-3 months to become significant while the GCL-IPL thickness remained unchanged (Figure 11)

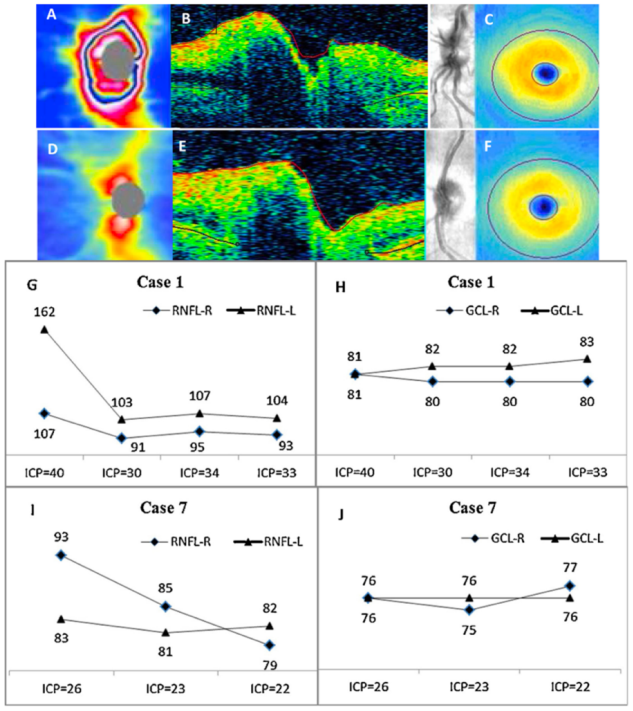


Figure 11 Thickness changes of RNFL and GCL=GCL–IPL (μm) in relation to intracranial pressure (ICP, cm H_2O) from case 1 and case 7 with IIH. Thickness maps (A and D) and B-scan of RNFL around ONH (B), and GCL–IPL thickness map (C) from the left eye of case 1 who had severe papilledema and CSF pressure of 40 cm H_2 O. Thickness maps (D) and B-scan (E) of RNFL around ONH, and GCL–IPL thickness map (F) from the left eye of case 7 who had hyperemia and CSF pressure of 26 cm H_2O. After removing CSF, thickness of RNFL (μm) fluctuated over time in both case 1 (G) and 7 (I). Thickness of GCL–IPL (m) remains stable irrespective of ICP changes in both case 1 (H) and 7 (J). From Paper II, reproduced under a CC-BY-ND-4.0 license, creative commons.

4.3 Paper III

Clinical outcomes

Data were obtained from 116 eyes of 60 subjects. 77% of eyes were emmetropes with spherical equivalent (SE) of 0±2 diopters (D), while the mean axial length for the study population was 23.86 mm (range 22.91–26.82 mm). The age, axial length, spherical equivalent, and visual acuity were similar between both sexes (P>0,05).

OCT data of macular ganglion cell layer

About one-quarter of all automated OCT segmentation needed some manual correction, which varied from relocation of the ETDRS circle to minimal adjustment of the segmentation of the GCL thickness or even along the entire B-scan. The thickest sectors in both sexes were S1, I1, N1 followed by T1 respectively, while the thinnest sector apart from C was I2 (see Figure 8 for definition of sectors). GCL thickness was not different in males compared to females except at the GCL-I2 and the average outer ring thickness (P<0.05), but after adjusting for age and axial length there were more sectors with significant differences between males and females (P<0.05). All mean sectors superior (SS), inferior (II), nasal (NN) and temporal (TT) were thicker in males compared to females after adjusting for age and axial length.

The total GCL volume was significantly thicker in males (1.13± 0.07 $mm^{3)}$ relative to females (1.09 ± 0.09 mm^3) after adjusting for axial length and age (P=0.031). There was a clear and significant thickness and volume reduction with age for the entire study population; after adjusting for sex and axial length, there was still a noticeable trend of reduction, but this was not statically significant. Analysis of the changes with age for both sexes separately showed that the GCL volume did not vary with age for males

(Pearson r=0.108, P=0.45); however, it was significant for females (Spearman r=-0.404, P<0.001) (Figure 12).

Bulbs with longer axial length had a thinner GCL which was significant even after adjusting for age and sex (P=0.048) (Figure 13).

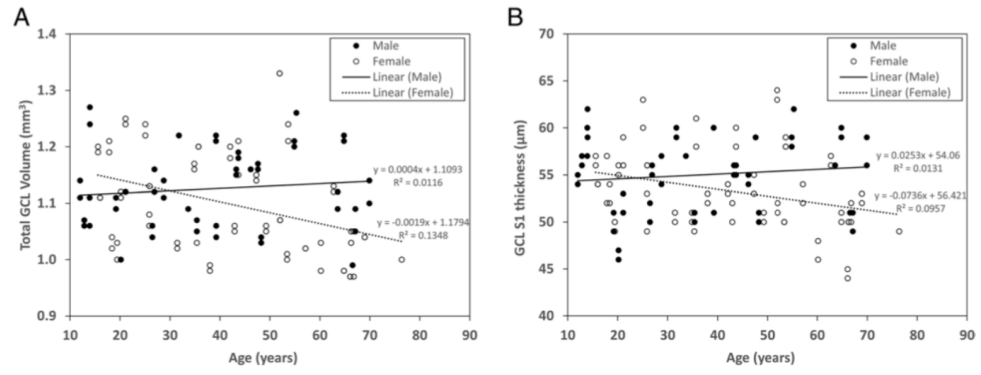


Figure 12 Variation of total GCL volume (A) and GCL S1 segment (B) with age in the entire study population, stratified by sex. Each data point represents a single eye. The regression line and equations are shown. After adjustment for axial length and sex, total GCL volume did not have an age association (P = 0.138); however, the association with age differed for females and males, being significant for females only (P = 0.003) From Paper III, reproduced under a CC-BY-ND-4.0 license, creative commons.

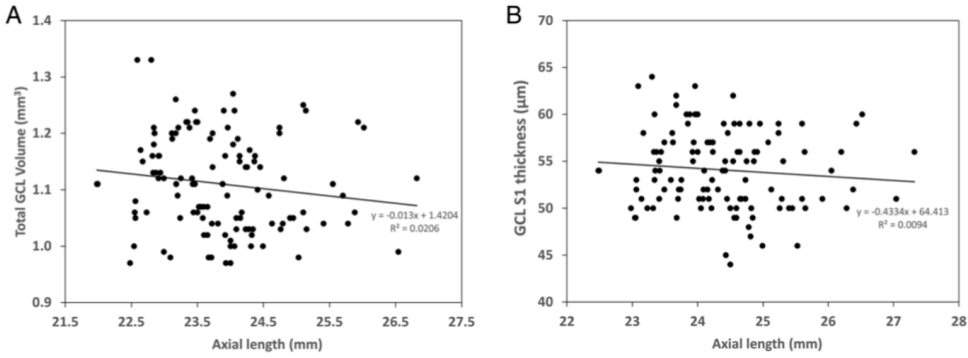


Figure 13 Variation of total GCL volume (A) and GCL S1 thickness (B) with axial length in the entire study population. Each data point represents a single eye. The regression line and equation are shown. After adjustment for age and sex, reduction in total GCL volume was associated with an increase in axial length (P = 0.048) From Paper III, reproduced under a CC-BY-ND-4.0 license, creative commons.

4.4 Paper IV

Clinical outcomes

A total of 18 patients and 22 healthy control subjects completed the study. The healthy controls were younger than the BMS group (P=0.016). Optic neuritis was (ON) found in 8 BMS patients (44.4%). Only 4 patients received treatment for MS (first line) whereas the others remained without any treatment. The disease's duration was 24.7 (11-40) years and the highest EDSS score was 3 in one case at the final study visit.

OCT measures from BMS and healthy control group

OCT data was collected as peripapillary RNFL and macular GCL-IPL thickness. The RNFL and GCL-IPL in the BMS group (all eyes including optic neuritis eyes) were thinner than the healthy controls both at baseline and at final visit.

Annual rate of thinning of RNFL in µm did not differ between the groups after adjusting for age (P=0.569) while the annual rate of thinning for GCL-IPL was

significant for both groups before and after adjusting for age (P=0.016 and P=0.026 respectively).

The OCT measures for best eye (non-optic neuritis) tended towards thinner layers in the BMS group but this was not statically significant except for GCL-IPL at baseline and final visit (P<0.05). There was no significance in annual thinning rate of RNFL in (non-optic neuritis) eyes in BMS (-0.2(0.27) μm/year) and the healthy control group (-0.05 (0.3) μm/year) (P>0,05 after adjusting for both age and sex). The thinning rate of GCL-IPL showed a similar result for BMS (-0.19(0.15) μm/year) and (-0.0(0.11) μm/year) for the healthy group (P=0.292) and remained non-significant after adjustment for both age and sex (P>0.05). There was no correlation between thinning rate and disease duration in the BMS group for both RNFL and GCL-IPL (r = −0.147, p = 0.560, C.I. 95% and r = −0.478, p = 0.061, C.I. 95%), respectively.

5. Discussion

The uniqueness of ganglion cell structure and organization within the inner part of the retina and its connections to the brain presents a unique opportunity for this layer to be investigated by different methods. One of the easiest, and nowadays most accessible and informative methods is OCT. Recent advances in OCT technology giving the possibility of micrometer-level segmentation of different layers (Figure 14) and the use of precise follow up algorithms in various commercial devices makes it possible to detect early changes and future progressions that may affect these cells.

The overall purpose of the four studies in this thesis was to investigate whether the ganglion cell examination by OCT can reflect physiological and pathological changes in the eye and brain. For this purpose, the studies were designed to examine the GCL and RNFL and determine changes in its thickness and/or volume considering three physiological factors age, sex, and axial length (Paper III), and three pathological conditions: inflammation (Paper I), compression (Paper II) and effect of a neurological disease without direct involvement of the optic nerve (Paper IV).

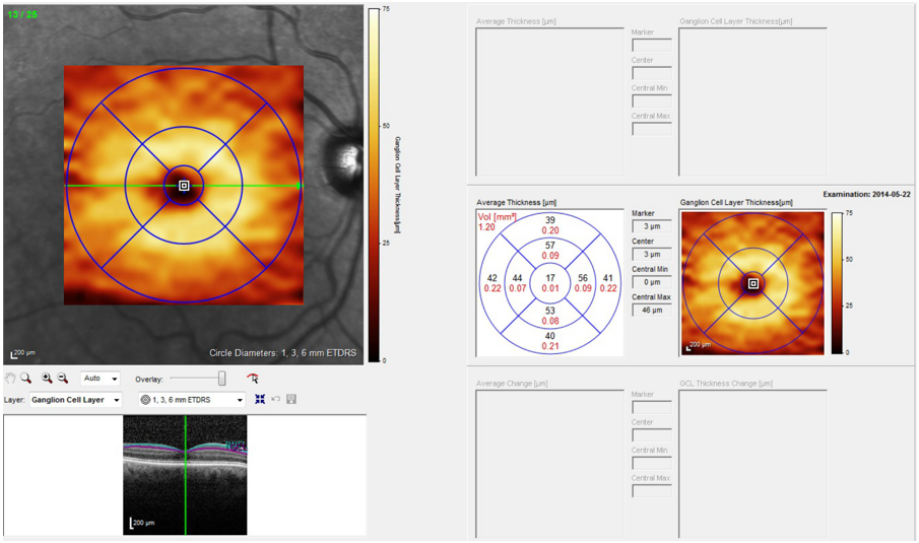


Figure 14 Automated segmentation and GCL thickness map from a healthy subject by HRT Heidelberg OCT of a healthy subject.

5.1 OCT changes in optic neuritis (Inflammation) Paper I

The OCT measurement of peripapillary RNFL (Retinal Nerve Fiber Layer) showed a thickening early at the acute stage of optic neuritis which is in line with what is already known (Costello et al. 2006; Syc et al. 2012; Toosy et al. 2014) as the axons, and not the soma, of the ganglion cells are the first target. The thickened RNFL layer showed gradual thinning during the follow-up period, up to 6-9 months after the incident. This thinning process is composed of two parts, the first part is the pseudo- thinning because it is not a real loss of RNFL but rather reduction in the swelling caused by the underlying pathology. Later, this is followed by an actual thinning in the RNFL due to

axonal loss but with no clear-cut distinction between the pseudo-thinning and the actual thinning of this layer.

On the other hand, inflammation in the optic nerve caused no swelling in the macular ganglion cell but instead a gradual reduction in the thickness of the ganglion cell layer after the acute incident subsided; the GCL is usually affected in cases of inflammation and Multiple Sclerosis (Saidha et al. 2013). As early as a few weeks after the acute optic neuritis we could measure thinning in the CGL-IPL layer, which was evident in all cases, both in the case of retrobulbar and intra ocular optic neuritis, occurring within 3 months in all patients.

The delay in actual thinning in RNFL makes the follow up measurements of this layer less reliable to reflect real loss as it exhibits pseudo-thinning while the GCL-IPL layer showed an early reduction in thickness even in the presence of optic disc swelling in the case of intrabulbar neuritis, which stabilized as early as 3 months. The GCL-IPL layer thus gives a more reliable and earlier window to monitor optic neuritis sequelae. As it is well known that retrobulbar neuritis gives a normal fundus appearance (Guy V. Jirawuthiworavong 2022) while on OCT examination we could see a clear thickening in the peripapillary RNFL which may add another layer of assessment of the optic disc in acute cases of sudden visual loss.

Another important observation with clinical implication is in the follow up of patients with optic neuritis. The usual clinical examination of visual acuity and visual field is subjective, and they usually normalize to 0.5 decimal or better in more than 95% of cases (Sabadia et al. 2016) after a few weeks to months. The VEP (Visual Evoked Potentials) is an objective test to detect residual optic nerve dysfunction post optic neuritis (You et al. 2011) as in our study (Paper I) all cases had a prolonged P100 latency. VEP examination is reliable but not easily accessible. OCT examination of the RNFL and ganglion cell layer gives another easily accessible and reliable alternative for

objective follow up of cases with optic neuritis and even in differentiating cases of suspected visual impairment because of optic neuritis from other causes.

5.2 OCT changes in IIH (compression) Paper II

The axons of the ganglion cells enter the optic disc, comprising most of its structure along with central retinal vessels and support tissue. Different OCT –optic disc parameters such as neuro-retinal rim thickness, cup volume and pigment epithelium central limit-inner limit of the retina minimal distance were investigated in different diseases, in particular in glaucoma (Sandberg Melin et al. 2019; Yang et al. 2017).

In Idiopathic Intracranial Hypertension (IIH), the intracranial pressure (ICP) compresses the optic nerve and affects the axoplasmic transport in the ganglion cell axons, leading to stagnation. This will result in swelling of the axons and leakage of the fluid, which may be noticed as swelling of the nerve fiber layer and clinically as optic disc swelling or edema. As the compression is behind the bulb it may need some time before it becomes evident as papilledema, which may explain why some cases of IIH have distention of the perioptic subarachnoid space but without papilledema (Favoni et al. 2018).

Swelling of the nerve fibers leads to thickening of the neuro-retinal rim and consequently reduction in the size of the optic cup; these are the first targets of elevated ICP in the eye. In Paper II, all IIH cases had long lasting disease with different grades of disc swelling from absent to mild to moderate and severe. All measurements of the ONH were abnormal compared to the control group. The IIH cases had a thicker rim and larger rim area ($P<0.05$) and smaller cup volume and lesser cup-to-disc ratio ($P<0.05$) despite similar disc area ($P>0.05$). On the other hand, there was no significant difference in the peripapillary RNFL or GCL-IPL thickness compared to the

healthy group. This may indicate that the swelling of the RNFL is confined to the ONH and does not reach the ring where the OCT measures the RNFL (at 3.46 mm for the Cirrus OCT) and as the patients were on treatment the ICP was not significantly elevated to cause damage to the GCL, which is seen in advance cases of IIH (Athappilly et al. 2019).

Moreover, longitudinal follow up of the two cases showed a reduction in the RNFL thickness after CSF tapping while the GCL-IPL remained normal. This indicates that even if the RNFL thickness was within normal ranges (according to the manufacturers normative values) there was likely a mild thickening in this layer. As the normative OCT reference, however, is overly broad, mild changes may be interpreted as being within normal values. The wide range of normal values may be due to many factors such as poor image quality, scan artifacts, ethnicity, axial length, refraction errors, sex, and variation in the number and size of central retinal vessels emerging from the optic nerve head (Budenz et al. 2007; Hardin et al. 2015; Lee et al. 2018; Ocansey et al. 2020). The normal GCL-IPL could reflect the benign course as all patients had normal visual acuity, color vision and no remarkable visual field defects despite visual symptoms and variation in ICP.

In summary, the OCT could detect changes in the optic nerve head, RNFL layer and GCL and aid in the follow up of patients with IIH.

5.3 OCT detection of physiological effects on GCL (Paper III)

Differences in GCL thickness in different anatomical sectors in the macula could be detected using the SD-OCT. The thickness difference in different sectors around the fovea reflects the embryological and developmental mobilization and displacement of ganglion cells at this region to devote the central fovea from retinal cells apart from

outer segments of photoreceptors. The thickest sector for the GCL was the superior sector followed by the inferior, nasal and temporal within the parafoveal zone (3mm ring). This indicates that the displaced cells move mainly in the vertical direction surrounding the fovea and settle in the parafoveal region vertically while the cells in the horizontal direction may spread more to the perifoveal zone, which is also noticed as the thickness of the nasal and temporal sectors of the outer ring are greater than the inferior and superior sectors.

Clinically, these findings are relevant as it is well known that the peripapillary RNFL is thicker in the vertical direction (superior and inferior) than in the horizontal direction (nasal, and temporal)(Pawar et al. 2014; Wu et al. 2023). The topography and thickness of the GCL can also reflect the GCL loss stages in glaucoma detected by visual field defects, as these changes are detected primarily in the paracentral regions, and the central field is the last to be affected (J. M. Kim et al. 2015) which made the GCL thickness an important factor to be studied in glaucoma progression (N. R. Kim et al. 2010; Sung et al. 2014) and even as a possible sensitive tool for glaucoma screening (Zhang et al. 2016).

The fact that the OCT detected thinning in the GCL volume with longer axial length bulbs may be explained by the distribution of a nearly fixed number of ganglion cells over larger retinal area in longer eyes with greater AL.

The OCT measurements indicated a reduction in GCL volume with age in the study population and although this was not statistically significant after adjusting for axial length and sex, there was still a noticeable thinning. As many studies showed that even the brain size including both the white and grey matter is reducing with age (Fujita et al. 2023; Peters 2006; Sele et al. 2021) the thinning of the GCL follows the same trend as the aging brain.

With the OCT, one could detect a difference in the total GCL volume between males and females. A thicker GCL was found in males than in females even after adjusting for axial length and age; according to the segmentation the difference was most significant in the inner 3mm. This is also a known trait in brain volume as males have a larger brain volume compared to females (Ruigrok et al. 2014).

Moreover, a separate analysis of the reduction in GCL volume with age in both males and females showed a higher rate of thinning in females than in males which is also noticed in brain size and volume changes between both sexes (Allen et al. 2003), especially in the occipital lobe (Takahashi et al. 2011). A possible explanation for the differences in ganglion cell volume is the sexual hormonal effect (both during the fetal stage and even in early infancy) on the neural development as well as axonal and process formation, as these hormones affect the brain in the same manner (Amateau et al. 2004; Peters 2006).

5.4 OCT detects benign course of Multiple sclerosis (Paper IV)

The RNFL and GCL-IPL thickness measurement is thoroughly investigated in MS (Britze and Frederiksen 2018; Frohman et al. 2006; Lidster and Baker 2012; Petzold et al. 2010). Benign multiple sclerosis (BMS) is no longer considered as a subtype of MS, and all MS subtypes are recommended treatment including clinically isolated syndrome (CIS) (Montalban et al. 2018). The aim of the treatment in MS is not to cure the disease but rather to reduce neurological disability caused by it (Cross and Riley 2022). BMS is known for its benign course where the patients keep mobile with less disability after many years of the disease (Glad et al. 2006) without treatment. In a cross-sectional study of the RNFL and GCL-IPL it was noticed that the BMS group had

a similar thinning rate compared to healthy population, that differed from patients with Relapsing-Remitting MS (RR-MS)(Y. M. Huang-Link et al. 2015).

The longitudinal measurement of the average thickness of peripapillary RNFL and macular GCL-IPL with OCT in non-optic neuritis (non-ON) eyes of the BMS group showed a similar annual thinning rate compared to the healthy control group ($P>0.05$). Regarding the peripapillary RNFL, we used the average thickness in our study as there is a variation in thickness of the RNFL because of the peripapillary vessels (Resch et al. 2015).

The thinning rate of both RNFL and GCL-IPL in the BMS group was not related to the disease duration ($r = -0.147$, $p = 0.560$, C.I. 95% and $r = -0.478$, $p = 0.061$, C.I. 95%) respectively. The decision to focus on the non-ON eyes was taken to isolate the general effect of the MS as a chronic autoimmune disease that affects the central nervous system from the direct impact of inflammation affecting the optic nerve in eyes with optic neuritis and to see whether the OCT examination can reflect this general effect. The OCT measurements also indicated that the non-ON BMS eyes tended to have a thinner retinal layer compared to the control group (although not significant $P>0.05$). This also reflects that the disease has a general mild, subclinical, effect on these layers, which is shown previously in postmortem studies (Ikuta and Zimmerman 1976). In summary, by using the OCT, significantly thinner layers in ON eyes could be identified; thinner but not statistically significant (clinically important) layers were found in non-ON reflecting subclinical impact, and a non-significant thinning rate with no or first line therapy compared to healthy population was found, indicating a benign course.

This similarity in thinning rate may be a criterion indicating the possible benign course in MS and furthermore the OCT as a tool could be used to identify subgroups of this devastating disease which may not require treatment.

5.5 Strengths and Limitations

The finding in Paper I where the GCL-IPL changes could be observed earlier than RNFL changes has a particularly important clinical significance. Despite the small number of subjects, the results were consistent in all cases. Strengths of Paper III were a broad exclusion criterion, correcting for refractive errors and adjustment for the variables of sex, age and axial length using the mixed linear model. The unique cohort of BMS patients with a natural MS course and without treatment/first line treatment, and the long duration of longitudinal measurement of the thinning rate are strengths of Paper IV. The small populations in the studies and the lack of a group with RR-MS in Paper IV are limitations for the studies. On the other hand, a general limitation with OCT examination of the GCL is that it is confined to the macular region where only specific types of ganglion cells type mainly reside.

6. Clinical relevance and future perspectives

The results and outcomes of the studies in this thesis as well as related prior research shows that examining the ganglion cells and its axons with OCT in different diseases gives another layer of reliable information that could be used to diagnose, follow up and to give insights into the prognosis of neurological diseases. The OCT examination is accessible to almost all patients visiting ophthalmology departments as well as many neurology departments, it is easy to operate with no contact, harm or discomfort to the patient and is a very cost-effective examination. The examination reflects different physiological changes in the retina that are correlated with changes in the brain. OCT parameters could be used as a biomarker for disease activity as in MS or as a biomarker of pathological changes in the brain demonstrated by retrograde degenerations as in cases of CVA (cerebrovascular accident) (Y. Huang-Link and Petré 2015) or in MS (Y. M. Huang-Link et al. 2014). Many prior studies have focused on the peripapillary RNFL thickness measurement but in this thesis, we could show that adding data about the GCL thickness will strengthen the use of OCT and adds valuable data to the clinical findings as in cases of optic neuritis and Idiopathic Intracranial Hypertension.

As was demonstrated in this thesis, the OCT examination of GCL and RNFL could be used as a cross sectional examination at the time of presentation to detect pathologies as in cases of acute optic neuritis, but a longitudinal follow up of the patient may be of clinical importance especially in cases of chronic diseases or even to investigate possible complications as in cases of MS or IIH. The role of OCT is expanding in both ophthalmology and neurology fields as it is used increasingly to examine many diseases that were previously considered to be solely a brain disease as in Parkinson's, Alzheimer's (Cunha et al. 2016; Paquet et al. 2007) , alcohol intake (Orum and Kalenderoglu 2020; Ozsoy and Alim 2020); and other diseases as

alcohol-associated optic neuropathy (Moura and Monteiro 2010), and methanol intoxication (Hlusicka et al. 2020).

The OCT could be used to detect changes that may be considered preclinical or subclinical as demonstrated in one of our studies (Paper IV). This presents the possibility to detect early changes in subjects with substantial risk to develop certain diseases or even to use the OCT as a screening tool in high-risk populations.

Detecting GCL changes with OCT combined with other clinical findings may indicate specific ganglion cell type damage or impact, as for example, the midget cells are mostly located centrally in the retina and are responsible for color vision while parasol cells are located around the fovea and are mostly responsible for contrast sensitivity. OCT findings may indicate specific disease suspicion as there are many diseases that affect specific types of ganglion cells. For example, in glaucoma it is well known that midget cells are affected first, and by OCT examination it is possible to detect GCL thinning before visual field defects, as shown (Kerrigan-Baumrind et al. 2000; Medeiros et al. 2013; Zhang et al. 2016). It is known that these cells respond better to motion rather than static stimuli which may indicate why frequency doubling test (FDT) perimetry is favored in early glaucoma over static automated perimetry (SAP) (Brusini and Busatto 1998; Lamparter et al. 2012), while in neurological diseases some studies showed that despite deep scotomas on the SAP visual field, the FDT perimetry was not affected (Fong et al. 2003) which may indicate that a specific type of RGC other than parasol cells may be affected in these cases.

On the other hand, the overlapping of receptive field of ganglion cells may be a challenge for precision detection of early changes in RGCs and even the overlapping of different RGC cell types may add another layer of complexity.

This thesis focused on using SD-OCT to detect changes in the retinal ganglion cells and its axons but with the continuing advances in OCT technology, the possibility

of even more precise examination with sub-micrometer levels could be achieved in the next generation OCTs, while adding other technologies as SLO and adaptive optics may enable cellular level examinations which gives another window into disease detection and follow-up . Another possible field of research is the reflectivity of different layers as this may change even prior to detectable changes in the thickness of the layer when examined with OCT (X. R. Huang et al. 2011; Meleppat et al. 2019). But regardless of the particular parameter measured, our work (Paper III) has shown that it is important to correct for axial length, sex, and age as potential cofounders that may affect any OCT examination of the GCL and RNFL.

Different OCT manufacturers have different examination standards and different normative values which may make comparing data from different OCT devices clinically inappropriate; by producing a software solution that may unify these results for the same object we may reach a milestone of comparing results from different devices. Moreover, anatomical challenges in case of peripapillary vascular thickness is a major issue in detecting a measurement that reflects the real thickness of the RNFL and not the vascular component. This is another area where more research is required. Finally, by combining OCT measurement of the RNFL thickness and vascular volume by OCT-angiography in the future, we may reach more accurate measurements that reflect the actual layer thicknesses.

7. Conclusions

In conclusion, OCT examination of ganglion cells and their axons could reflect pathological processes caused by different mechanisms such as inflammation, increased intra-cranial pressure and compression as well as a chronic neurological autoimmune disease that affects both the brain and eye. The examination of RGCs could provide a possible biomarker for severity of neurological diseases and may reflect severity of the disease. OCT examination of RGCs could reveal physiological changes in the eye that may mirror similar physiological changes in the brain.

To summarize, examination of retinal ganglion cells using OCT can reflect pathological and physiological changes in the eye and brain.

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