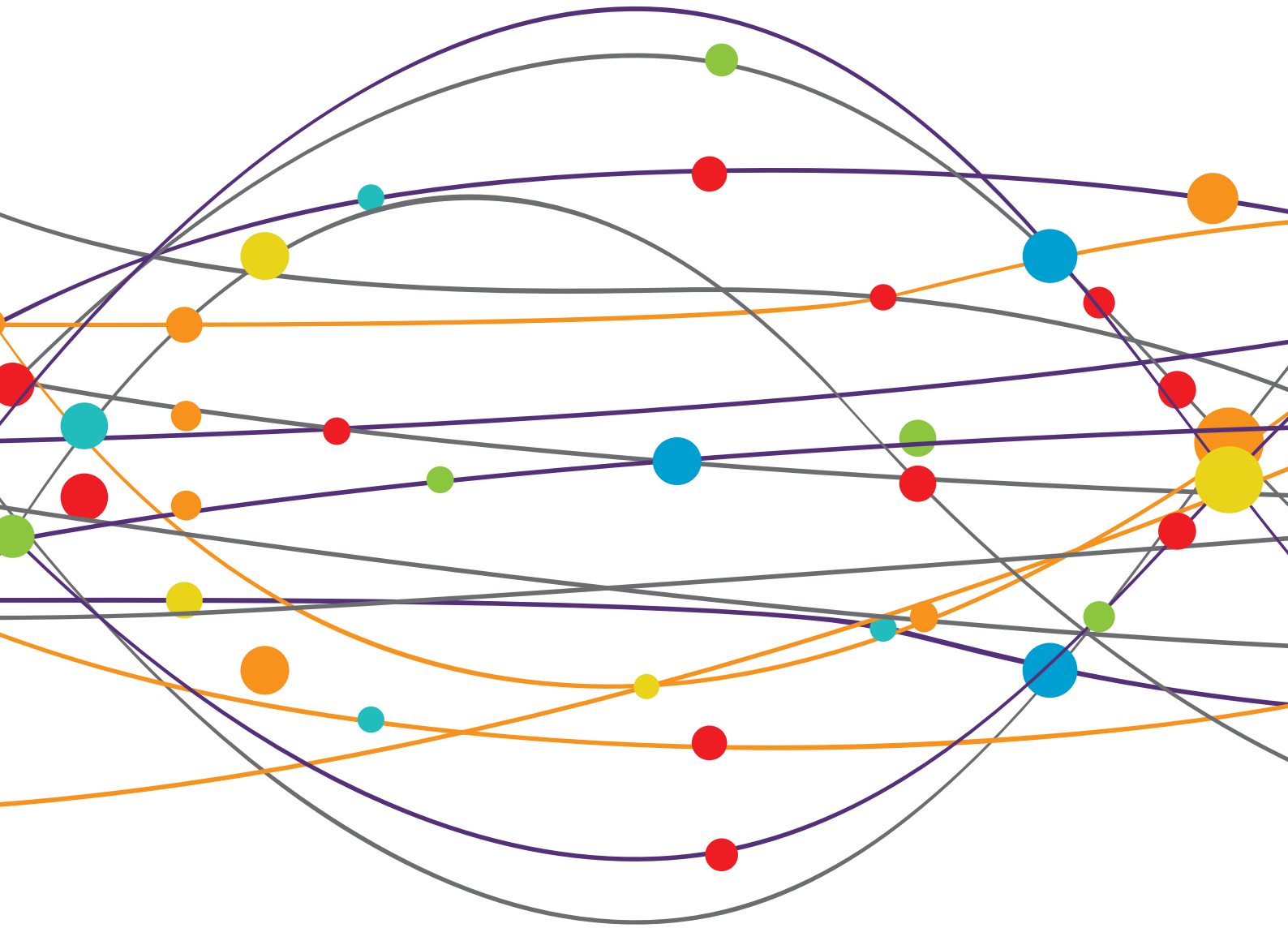
# BEHOLD THE EYE IN PARKINSON'S DISEASE & ALZHEIMER'S DISEASE

EDITED BY: Ivan Bodis-Wollner, Nicolás Cuenca and Raymond Chuen-Chung Chang
PUBLISHED IN: Frontiers in Neurology and Frontiers in Neuroscience



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ISSN 1664-8714
ISBN 978-2-88963-434-7
DOI 10.3389/978-2-88963-434-7

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# BEHOLD THE EYE IN PARKINSON'S DISEASE & ALZHEIMER'S DISEASE

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There are increasing lines of evidence showing that neurodegeneration in Alzheimer's disease (AD) and Parkinson's disease (PD) is not limited to the brain but also occurs in the retina. Consequently, AD/PD patients can gradually develop vision problems. This neurological and ophthalmological disorder creates a pressing need for developing therapy to treat vision impairment in AD/PD. On the other hand, pathophysiological changes in the retina may reflect what might happen in the same diseases in the brain. Thus retinal studies may allow us to develop quantifiable measures for the diagnosis and prognosis of disease progression. Furthermore, parallel or early pathophysiological changes of the retina in AD/PD allow us to study retina-brain interactions.

Several research groups have made advances in understanding pathophysiological changes of the retina in the eyes in AD/PD. It is the time to re-evaluate this issue. The aim of this Research Topic is to make collective effort to review the progress in this newly emerging multi-disciplinary field, to stimulate more research in AD/PD and Ophthalmology communities. The aim of this Research Topic is not simply to review, but also to foster cross-fertilization among neurobiologists, ophthalmologists, optometrist, neurologists and psychologist investigating vision cognition toward developing translational neuroscience of the retina in neurodegenerative diseases of the brain.

**Citation:** Bodis-Wollner, I., Cuenca, N., Chang, R. C.-C., eds. (2020). Behold the Eye in Parkinson's Disease & Alzheimer's Disease. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-434-7

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frontiers in Neurology

OPINION
published: 03 November 2015
doi: 10.3389/fneur.2015.00232

# Pathogenic microRNAs common to brain and retinal degeneration; recent observations in Alzheimer's disease and age-related macular degeneration

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**Keywords: complement factor H, disease heterogeneity, innate-immune and inflammatory response, miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, miRNA-155**

OPEN ACCESS

***Edited by:***
*Raymond Chuen-Chung Chang, The University of Hong Kong, Hong Kong*

***Reviewed by:***
*Kin Chiu, The University of Hong Kong, Hong Kong*

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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *16 August 2015*
***Accepted:*** *20 October 2015*
***Published:*** *03 November 2015*

***Citation:***
*Hill JM, Pogue AI and Lukiw WJ (2015) Pathogenic microRNAs common to brain and retinal degeneration; recent observations in Alzheimer's disease and age-related macular degeneration. Front. Neurol. 6:232. doi: 10.3389/fneur.2015.00232*

MicroRNAs (miRNAs), ~22 nt single-stranded non-coding RNAs (ncRNAs) abundant in the human brain and retina, have emerged as significant post-transcriptional regulators of messenger RNA (mRNA) abundance and complexity in the human central nervous system (CNS) in aging, health, and disease. Of the 2050 different miRNAs in the human body so far identified, only about 25–30 are abundant in either the brain or the retina, underscoring the high selection pressure carried by RNA sequences located within these select ncRNAs (1–7). It is noteworthy to point out that: (i) that brain neocortex and retina share a common neuroectodermal origin; (ii) that brain and retina share a subfamily of specific miRNA species; and (iii) that the multilayered assemblies of both neural and retinal cells are targeted by pathogenic processes that drive progressive pro-inflammatory neurodegeneration (5–9). Indeed, pathologically up-regulated miRNAs common to both the prototypic age-related inflammatory degeneration of the brain in Alzheimer's disease (AD) and of the retina in age-related macular degeneration (AMD) appear to be associated with deficits in the expression of messenger RNA (mRNA) and gene families involved in the innate-immune response, inflammation, neurotrophism, synaptogenesis, and amyloidogenesis (**Figure 1**). In this "Opinion" paper for the *Frontiers in Neurology Special Research Topic*, we will highlight some of the most recent work in this research area, with emphasis on a family of five up-regulated pro-inflammatory miRNAs – miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155 – that are emerging as key mechanistic contributors to the AD and AMD process.

Homeostatic levels of specific miRNAs are natural indicators of neurological health of both the brain and retina (2–10, 31). Recently, multiple independent neurological research laboratories have provided evidence for the up-regulation of a small group of five inducible miRNAs in age-related diseases involving a progressive inflammatory degeneration. That these five miRNAs – miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155 – are up-regulated in both AD and AMD underscores the concept that the brain and retina share common pathological signaling of a pre-existing subfamily of miRNAs that individually contribute to various aspects of neurodegenerative

**Abbreviations:** AMD, age-related macular degeneration; AD, Alzheimer's disease; CFH, complement factor H; miRNA, microRNA

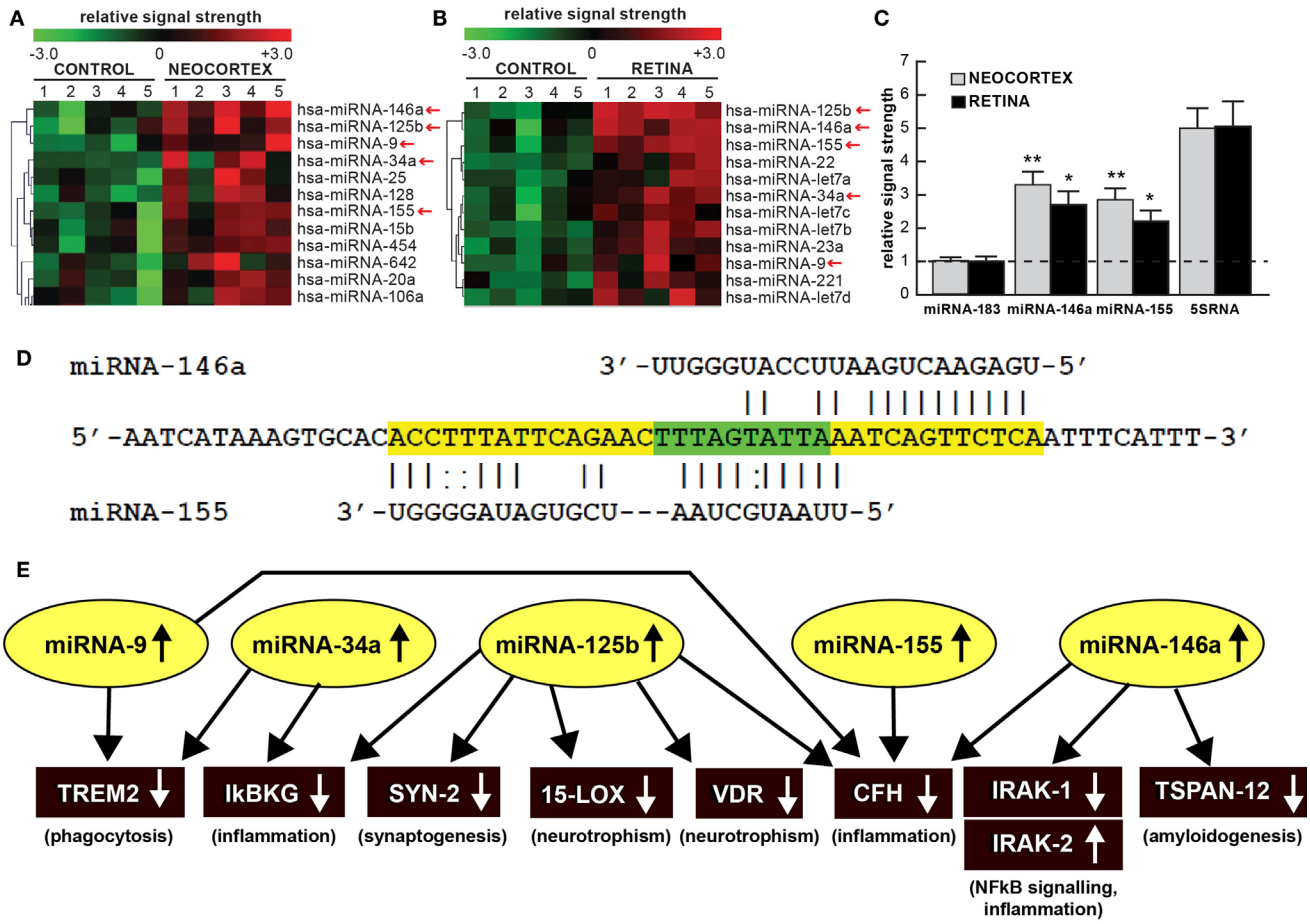


**FIGURE 1 | (A)** Color-coded cluster analysis of significantly up-regulated microRNAs (miRNAs) in the neocortex of AD ($N = 5$) versus age-matched controls ($N = 5$) and **(B)** in the whole retina of AMD ($N = 5$) versus age-matched controls ($N = 5$); this small family of "pro-inflammatory" miRNAs consisting of miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155 are often found to be up-regulated approximately twofold or more over controls (small red arrows); interestingly these miRNAs are inducible and under transcriptional control by NF-kB [**Figure 1** adapted from Ref. (10–13)]; the relative expression levels for two sequence-related microRNAs, miRNA-146a and miRNA-155 the brain neocortex and retina are shown in **(C)** relative to levels for an unchanging brain and retinal control miRNA-183, which was set to 1.0 and marked by a dashed horizontal line; another abundant control signal is 5SRNA with a relative signal strength of ~5.0 (shown at ~1/20th of its actual abundance in the brain and retina; see text) (12, 13). In these samples, all control and AD neocortical samples were obtained from the superior temporal neocortex (Brodmann area A22); control and AMD retinal samples were obtained from whole retina; all control, AD and AMD samples had post-mortem intervals (PMI; death to brain freezing interval) of 2 h or less (2, 9, 10). Controls were age-matched to moderate-to-late stages of AD or AMD; increases in specific miRNAs increased as disease stage advanced [Ref. (11, 12, 14–17); data not shown]; further details on the pathology of these samples have been recently published (11–20). There were no significant differences in age, PMI, or RNA yield or quality between either the brain or the retinal tissues. Of the 12 different homo sapien micro-RNAs (hsa-miRNAs) shown, miRNA-146a and miRNA-155 exhibited the most consistent up-regulation compared with age-matched controls (*$p < 0.05$; **$p < 0.01$, ANOVA); **(D)** the 3′UTR of the mRNA of complement factor H (CFH); a major regulator of the innate-immune and inflammatory response, see text; [(21, 22)] is a prime example of brain and retinal gene expression regulation by multiple and common miRNAs – miRNA-146a and miRNA-155; **(D)** shows the complementarity map between miRNA-146a or miRNA-155 and part of the 232 nt CFH 3′UTR sequence. Overlapping miRNA-146a and miRNA-155 high-affinity binding sites in the CFH mRNA 3′UTR (each has an energy of association of less than −22 kcal/mol) that defines an exceptionally stable miRNA–mRNA interaction and a potentially common CFH mRNA 3′-UTR miRNA regulatory control region 5′-TTTAGTATTAA-3′ (overlaid in green; see text) (12, 13, 23); we cannot exclude the participation of other human brain- or retina-enriched miRNAs or other small ncRNAs which may additionally contribute to the neuropathological mechanisms of AD or AMD pathology; **(E)** taken together, these recent findings in part define a highly interactive network of NF-kB-sensitive, up-regulated miRNAs in diseased brain and retina that can explain much of the observed pathology associated with AD and AMD. The CNS-abundant, miRNA-125b is a central member of this up-regulated miRNA group that may be in part responsible for driving deficits in phagocytosis (triggering receptor expressed in microglial cells; TREM2), innate-immune signaling and chronic inflammation (IkBKG, CFH), impairments in neurotransmitter packaging and release (synapsin-2; SYN-2), and neurotrophism (15-lipoxygenase, vitamin D receptor; 15-LOX, VDR). Other NF-kB-sensitive up-regulated miRNAs (such as miRNA-146a) appear to be responsible for the observed deficits in NF-kB regulation (IRAK-1, IRAK-2) and/or amyloidogenesis (tertraspanin 12; TSPAN12); these up-regulated miRNAs and down-regulated mRNAs form a highly integrated, self-perpetuating pathogenic miRNA-mRNA signaling network due to chronic re-activation of NF-kB stimulation perhaps through the involvement of deficits in IkBKG signaling (10–13). Inhibition of the NF-kB initiator or individual blocking of the pathogenic induction of these five miRNAs may provide novel therapeutic benefit for the clinical management of AD or AMD, however what NF-kB or miRNA inhibition strategies and/or protocols, or whether they can be utilized either alone or in combination, remain open to investigation (17, 19, 20, 24, 25). Extensive recent data in human brain cells in primary culture have indicated that these approaches may neutralize this chronic, inducible, progressive pathogenic gene expression program to re-establish brain and retinal cell homeostasis, and ultimately be of novel pharmacological use in the clinical management of AD and/or AMD (19, 20, 26–30).

disease (5–12, 31–35). Accumulating evidence, including very recent research findings over the last 6 months indicate that each of these miRNAs share the following six features: (i) that they are basally expressed in control brain neocortex and retina (2–9); (ii) that *in vitro* they can be induced by a wide range of environmental- and inflammation-linked physiological stressors, including pro-inflammatory cytokines, amyloid beta (Aβ42) peptides, neurotoxic metal sulfates, and neurotropic viruses such as herpes simplex virus-1 (HSV-1) (12, 16, 17, 32–35); (iii) that this group of five pro-inflammatory miRNAs are over-expressed at least twofold in stressed brain or retinal cells and in AD or AMD affected tissues (14, 15, 32); (iv) that together, via down-regulation of multiple mRNA targets (and hence deficits in the expression of genes encoded by those mRNAs) they regulate various pathophysiological features characteristic of AD and AMD, including impairments in phagocytosis, synaptogenesis, neurotrophism, NF-kB signaling and stimulation of progressive inflammation and amyloidogenesis (**Figure 1**) (7, 12, 13, 23, 26–28, 36); (v) that all five of these pro-inflammatory miRNAs are under transcriptional control by NF-kB (chiefly the heterotypic p50/p65 dimer) in human primary neuronal-glial co-cultures, AD and AMD tissues (7, 11–13, 23, 26–28, 36, 37); and (vi) that both NF-kB inhibitors and anti-microRNAs (anti-miRs) effectively knock down their expression in human brain and retinal cell culture experiments, and may ultimately be of use therapeutically in the clinical management of AD or AMD (17, 18, 26–29).

Much of the recent research work emphasizing this commonality of the same miRNAs in basic pathological processes involving brain and retinal degeneration, as exemplified by miRNA profiling in AD, AMD, and transgenic AD or AMD (TgAD, TgAMD) models, has been summarized in **Figure 1** (5–10, 12, 14, 17, 25, 31–35). First, when compared to the unchanging 22 nt miRNA-183 and the 120 nt 5S ribosomal RNA (5S rRNA; 5SRNA) control markers, the five member pro-inflammatory microRNAs miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155 are found to be amongst the most consistently up-regulated miRNAs in both degenerating human brain neocortex (**Figure 1A**) and retina (**Figure 1B**). Of this group of five pro-inflammatory microRNAs, miRNA-146a and miRNA-155 are typically found to be increased ~2.5- to 3.3-fold over age-matched controls (**Figure 1C**). To add another layer of genetic complexity for post-transcriptional regulation, both miRNA-146a and miRNA-155 recognize an overlapping 3′ untranslated region (3′UTR) of the complement factor H (CFH) mRNA (highlighted in green; CFH loss-of-function mutations or CFH expression deficits are associated with both AD and AMD; see below; **Figure 1D**). Indeed, the up-regulation of these same five pro-inflammatory miRNAs (yellow ovals in **Figure 1E**) appear to form a highly interactive miRNA–mRNA network that can in part explain the down-regulation of specific brain and retinal genes (black rectangles) involved in phagocytosis, inflammation, synaptogenesis, neurotrophism, NF-kB signaling, and amyloidogenesis (**Figure 1E**; see also the legend to **Figure 1** wherein the details of this highly interactive network are further described).

Alterations in innate-immune signaling are a consistent feature of both AD and AMD (4, 5, 9, 15). The highly soluble, hydrophilic 155-kDa glycoprotein CFH is one very illustrative example of an innate-immune repressor and complement control protein whose abundance and/or activity is significantly down-regulated in both AD and AMD [(9, 15, 21, 22, 35); see **Figure 1D**]. CFH (chr 1q32; also known as AC3bINA, adrenomedullin binding protein-1, AM binding protein-1 factor H, β1H globulin, H factor, and H factor-1) is an important member of the regulator of complement activation (RCA) group of proteins encoded within the RCA gene cluster and normally performs a systemic sentinel function against unscheduled or spontaneous immune system activation (9, 15). CFH mRNA abundance is down-regulated in AD and/or AMD by a miRNA-146a- and/or miRNA-155–CFH–3′UTR-based complementarity mechanism and/or by a Y402H loss-of-function mutation (15, 21, 22). Hence an insufficiency in a homeostatic amount of functioning CFH (as down-regulated by miRNA-146a and miRNA-155) may have the same end result as the loss-of-function Y402H mutation in CFH (21, 22). It is important to note that CFH mRNA and hence CFH gene expression appears to be down-regulated by at least two different miRNAs – miRNA-146a and/or miRNA-155 – and their differential recognition of overlapping binding sites in the human CFH mRNA 3′UTR may be dependent on yet-to-be-defined genetic factors and mechanisms characteristic of individual brain or retinal cells [**Figure 1D**; (9, 15, 21, 22, 35)].

In summary, it is our opinion that in miRNA research in human degenerative diseases including AD and AMD, several critical concerns have surfaced: (i) that brain and retinal miRNAs typically possess limited stabilities, however miRNA half-lives can be considerably extended via their sequestration into exosomes or the use of other protective strategies such as adsorption or tertiary folding into RNAse-resistant structures that may escape initial miRNA detection using traditional methods (17, 18, 23–25); (ii) that accurate quantification of miRNAs is technically feasible although it still remains challenging due to the small size of mature miRNA isoforms, adsorption to "inert" surfaces, high sequence homology amongst individual miRNAs, 5′ and 3′ end polymorphisms, spatial-temporal expression patterns and high dynamic range of miRNA expression (13, 17, 18, 24); (iii) that miRNA profiling in different AD or AMD studies suffers from a poor consensus regarding their abundance and complexity; the latter a very recently acknowledged concern in the field (4–7, 14, 17); and (iv) discrepancies of miRNA abundances in anatomical areas sampled, variations in patient drug history, the PMI of the AD and AMD patients and other factors. Together these constitute practical methodological challenges, especially in the realm of useful biomarkers and diagnostics for AD or AMD detection (3, 6, 17, 25, 34). Despite these recent concerns data has begun to filter through on the involvement of distinct miRNA families and miRNA–mRNA signaling networks linked to innate-immune system alterations, inflammatory, neurotrophic, and amyloidogenic consequences in AD and AMD. These have steadily yielded a deeper appreciation into the onset and propagation of complex miRNA–mRNA-modulated biological networks that directly underlie the pathogenesis of AD and AMD. Lastly, miRNAs are highly soluble and mobile, and are able to transverse plasma membranes either freely, adsorbed to carrier molecules or contained within exosomes (17, 19, 23, 25). That AD and AMD are both progressive "propagating"

disease entities suggest a potential "spreading factor" role for selective miRNAs in the cognitive and visual circuitry, an evolving research area in which specific combinations of miRNAs may be playing hitherto unrecognized pathogenic roles.

## ACKNOWLEDGMENTS

This work was presented in part (i) at the Alzheimer's Association International Conference (AAIC 2015) Annual Meeting 18–22 July 2015, Washington, DC, USA; (ii) at the Association for Research in Vision and Ophthalmology (ARVO) Annual conference 3–7 May 2015 in Denver, CO, USA; and (iii) at the Society for Neuroscience (SFN) Annual Meeting 15–19 November 2014, Washington, DC, USA; sincere thanks are extended to Drs. F. Culicchia, C. Eicken, and C. Hebel for short post-mortem interval (PMI) human brain and/or retinal tissues or extracts, primary miRNA array work and initial data interpretation, and to D. Guillot for expert technical assistance. Thanks are also extended to the many neuropathologists, physicians and researchers of Canada and the United States who have provided high quality, short post-mortem interval (PMI) human CNS and retinal tissues or extracted total brain and retinal RNA for scientific study. Research on miRNA in the Lukiw laboratory involving the innate-immune response in AD, AMD and in other forms of neurological or retinal disease, amyloidogenesis, synaptogenesis and brain inflammation was supported through an unrestricted grant to the LSU Eye Center from Research to Prevent Blindness (RPB); the Louisiana Biotechnology Research Network (LBRN) and NIH grants NEI EY006311, NIA AG18031, and NIA AG038834 (WL).

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 18 December 2015
doi: 10.3389/fnins.2015.00479

# Association Between Alzheimer's Disease and Glaucoma: A Study Based on Heidelberg Retinal Tomography and Frequency Doubling Technology Perimetry

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**Specialty section:**
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

**Received:** 27 July 2015
**Accepted:** 30 November 2015
**Published:** 18 December 2015

**Citation:**
Cesareo M, Martucci A, Ciuffoletti E, Mancino R, Cerulli A, Sorge RP, Martorana A, Sancesario G and Nucci C (2015) Association Between Alzheimer's Disease and Glaucoma: A Study Based on Heidelberg Retinal Tomography and Frequency Doubling Technology Perimetry. Front. Neurosci. 9:479. doi: 10.3389/fnins.2015.00479

**Aim:** To assess the frequency of glaucoma-like alterations in Alzheimer's disease (AD) patients using Heidelberg Retinal Tomograph III (HRT-3) and Frequency Doubling Technology (FDT) perimetry.

**Methods:** The study included 51 eyes of 51 AD subjects and 67 eyes of 67 age- and sex-matched controls. Subjects underwent an ophthalmological examination including measurements of intraocular pressure (IOP), Matrix FDT visual field testing, optic nerve head morphology and retinal nerve fiber layer thickness (RNFLt) assessment by slit-lamp biomicroscopy and HRT-3.

**Results:** The frequency of alterations was significantly higher in the AD group (27.5 vs. 7.5%; $p = 0.003$; OR = 4.69). AD patients showed lower IOP ($p = 0.000$) despite not significantly different values of central corneal thickness (CCT) between the groups ($p = 0.336$). Of all the stereometric parameters measured by HRT-3, RNFLt was significantly lower in AD patients ($p = 0.013$). This group also had significantly worse results in terms of Moorfields Regression Analysis ($p = 0.027$). Matrix showed significantly worse Mean Deviation (MD) ($p = 0.000$) and Pattern Standard Deviation (PSD) ($p = 0.000$) values and more altered Glaucoma Hemifield Test ($p = 0.006$) in AD patients. Pearson's R correlation test showed that Mini Mental State Examination is directly correlated with MD ($R = 0.349$; $p = 0.034$) and inversely correlated with PSD ($R = -0.357$; $p = 0.030$).

**Conclusion:** Patients with AD have a higher frequency of glaucoma-like alterations, as detected by the use of HRT-3. These alterations were not associated with elevated IOP or abnormal CCT values.

**Keywords: Alzheimer disease, glaucoma, optic nerve head, HRT-3, FDT, RNFL, CCT**

## INTRODUCTION

Glaucoma, the major cause of irreversible blindness worldwide, is a progressive optic neuropathy associated with degeneration of retinal ganglion cells (RGCs) and their axons, and it is characterized by a typical optic nerve appearance and corresponding visual field loss (European Glaucoma Society, 2008). Until now, increased intraocular pressure (IOP) has been considered the major treatable risk factor for the disease. Therefore, despite the relationship between the reduction of IOP and glaucomatous damage is not yet known, the achievement of an "individual" target pressure has paramount importance. However, it has been clinically observed that a significant reduction of the IOP does not always stop the disease (Leske et al., 2003). Some patients, in fact, experience a progression of glaucoma even after a significant reduction of the IOP levels, whereas others show pathognomonic alterations despite the IOP is in the normal range.

The exact pathophysiology underlying the glaucoma is currently unknown. However, studies using magnetic resonance imaging (MRI) have shown that the disease extends well beyond the eye, affecting the entire visual pathway, thus suggesting a possible connection with other neurodegenerative diseases (Nucci et al., 2013).

Interestingly, clinicians and researchers have observed close links between glaucoma and Alzheimer's Disease (AD) (Wostyn et al., 2009) whose importance is increasing as life-expectancy of populations rises.

AD is the leading cause of dementia worldwide and is estimated to affect approximately 50–60% of dementia patients. The disease is characterized by a gradual, progressive and irreversible decline in cognitive function and is associated with certain risk factors, such as genetics and vascular alterations. The presence of extracellular amyloid-β (Aβ) senile plaques and the intracellular deposition of abnormally phosphorylated tau protein are the main hallmarks of the disease (Blennow et al., 2006).

Although several studies have suggested that there is no association between glaucoma and an increased risk of developing AD (Kessing et al., 2007; Bach-Holm et al., 2012; Ou et al., 2012; Keenan et al., 2014) other population-based studies have reported a higher prevalence of glaucoma in patients affected by the disease (Chandra et al., 1986; Bayer and Ferrari, 2002; Bayer et al., 2002a,b; Tamura et al., 2006; Lin et al., 2014; Pelletier et al., 2014). These findings have been supported by data showing that inheritance of the AD-associated [epsilon]4 allele is twice as high among glaucoma patients, irrespective of the presence of ocular hypertension (Wostyn et al., 2009).

These studies may support the hypothesis that, in some patients, glaucoma could be the expression of a neurodegenerative process of the central nervous system that may be only partially influenced by ocular factors, such as the IOP (Nucci et al., 2013, 2015).

The aim of this study was to evaluate the frequency of glaucoma-like alterations in a group of patients with AD using diagnostic criteria based on Frequency Doubling Technology (FDT) perimetry and Heidelberg Retinal Tomography-3 (HRT-3).

## MATERIALS AND METHODS

This study adhered to the Declaration of Helsinki, and the Institutional Review Boards and Ethics Committees of Tor Vergata University Hospital approved its protocol.

Written informed consent was obtained from all participants.

The study included 51 consecutive newly diagnosed Alzheimer's disease cases (51 eyes), recruited from the Department of Neurology of Tor Vergata University Hospital.

AD diagnosis was made according to the NINCDS-ADRDA guidelines and the Diagnostic and the Statistical Manual of Mental Disorders (DMS IV) (McKhann et al., 1984; American Psychiatric Association, 2004). All patients underwent a complete clinical investigation, including medical history, neurological examination, mini mental state examination (MMSE), a complete blood screening (including routine exams, thyroid hormones, level of B12), neuropsychological examination (Pierantozzi et al., 2004) a complete neuropsychiatric evaluation and neuroimaging consisting of 1,5 T magnetic resonance imaging. All the patients were studied for ApoE genotype. Exclusion criteria were the following: (1) patients with isolated deficits and /or unmodified MMSE ($\geq$25/30) on revisit (6, 12, 18 months follow-up), patients with clinically manifest acute stroke in the last 6 months showing an Hachinsky scale >4, and a radiological evidence of sub-cortical lesions. None of patients revealed pyramidal and/or extrapyramidal signs at the neurological examination.

The control group consisted of 67 healthy subjects (67 eyes), who were randomly recruited from the General Outpatient Clinic of the Ophthalmological Department of Tor Vergata University Hospital.

All the enrolled subjects underwent a comprehensive eye examination, including the determination of best corrected visual acuity (BCVA) with logarithmic visual acuity charts "ETDRS" (Precision Vision, la Salle USA), IOP measurement using Goldmann applanation tonometry, central corneal thickness (CCT) measurement using an ultrasound pachymeter (Pachette DGH 500; DGH Technology, Inc., Philadelphia, PA), gonioscopy, and slit-lamp biomicroscopy of the anterior and posterior segments.

Subjects with spherical refraction beyond ±5.0 D and/or cylinder correction beyond ±3.0 D, or any ocular or systemic disease, which could affect the optic nerve or the visual field examination results, were excluded from the study.

Structural evaluation of the optic disc and retinal nerve fiber layer (RNFL) were performed using HRT-3 (Heidelberg Engineering, Heidelberg, Germany), which is a proven tool for detecting and managing glaucoma, assisting in the identification of pre-perimetric disease and monitoring of progression. In all astigmatic eyes beyond ±1.0 D, corrective cylindrical lenses were used. All participants whose tests revealed a Mean Pixel Height Standard Deviation >30 micron were excluded from the study. Disc measures of the enrolled patients did not exceed the database disc area cut-offs provided by the HRT-3 manufacturer.

(Dascalu et al., 2010) The same operator using only four points manually traced a contour line around the optic disc edge (the inner edge of Elschnig's ring). For each test Moorfields Regression Analysis (MRA), Glaucoma Probability Score (GPS) and the following stereometric parameters were evaluated: retinal nerve fiber layer thickness (RNFLt), Rim Area, Rim Volume, Cup-Shape Measure (CSM), Height Variation Contour (HVC), Cup/Disc Ratio (CDR) asymmetry, and vertical Cup/Disc Ratio (vCDR) (**Figure 1**).

MRA results, which differentiates abnormal from healthy optic nerve heads by detecting diffuse and focal changes of the neuroretinal rim area, were defined as follows: 1 = within normal limits; 2 = borderline; 3 = outside normal limits.

GPS, which automatically identifies patterns of structural change consistent with glaucoma providing a probability of abnormality, was defined as follows: 1 = within normal limits; 2 = borderline; 3 = outside normal limits; 0 = not classified.

The optic disc appearance was also assessed by slit lamp biomicroscopy examination and defined as pathological when at least one of the following features was present: vCDR asymmetry between the eyes ≥0.2, CDR ≥0.5, neuroretinal rim thinning, splinter-shaped disc hemorrhages, notching, localized pallor, focal, or generalized peripapillary atrophy or nerve fiber layer defects, baring of circumlinear or cilioretinal vessels (European Glaucoma Society, 2008).

Matrix FDT perimetry (Welch Allyn, Skaneateles Falls, NY, USA and Carl Zeiss Meditec, Dublin, CA, USA) was obtained from all participants using the 30-2 program. Before proceeding, all patients received a training and underwent a pre-test lasting 60 s. The examination was performed on each eye. Only subjects who performed reliable visual fields (≤33% fixation losses, false positives, and false negatives) were included. For each test the following index were evaluated: mean deviation (MD), pattern standard deviation (PSD), and glaucoma hemifield test (GHT). MD is a measure of the average deviation from the patient's light sensitivity and that of age-matched controls. PSD shows localized loss of light sensitivity, which is one of the hallmark of glaucoma. GHT warns the clinician about the occurrence of significant differences in terms of clusters of altered points between the superior and inferior hemifields (Scuderi et al., 2008). GHT results were defined as follows: 1 = within normal limits; 2 = limit; 3 = borderline; 4 = outside normal limits; 5 = general loss of sensitivity. The severity of visual field damage was classified according to the FDT Glaucoma Staging System 2 (GSS2) (Brusini, 2006).

The same operator throughout the whole study performed all the examinations. For each patient, only the eye with the worse results in terms of RNFLt, assessed by HRT-3, was included in the study. Glaucoma-like alterations were defined as the occurrence of visual field specific defects (GSS2 stage ≥1) and morphological optic disc alterations at biomicroscopy and/or pathological changes of HRT-3 parameters.

All data were initially entered into an EXCEL database (Microsoft, Redmond, Washington—United States) and the analysis was performed using the Statistical Package for the Social Sciences, Windows version 19.0 (SPSS, Chicago, Illinois, USA).

Gaussian distributions were differentiated from non-Gaussian ones by the use of the Kolmogorov-Smirnov test. Descriptive statistics consisted of the mean ± standard deviation (SD) for parameters with Gaussian distribution or frequencies (%) for occurrences. Gaussian parameters were analysed using one-way ANOVA test. Conversely, non-Gaussian parameters were analysed using Mann Whitney U or Kolmogorov-Smirnov Z tests. For categorical variables, comparison of frequencies among groups was performed using the Chi-Square test or Fisher's exact test. Correlations among ocular parameters (MD, PSD, GHT, MRA, RNFL, IOP) and MMSE were performed using Pearson's R correlation test. A $p$-value $< 0.05$ was considered statistically significant.

## RESULTS

Descriptive analysis of the AD and Control Groups is shown in **Table 1**.

All of the patients resulted E3/E4 in ApoE genotype study. No significant differences between the groups were found when considering sex (ANOVA One-way test; $p = 0.536$) and age (ANOVA One-way test; $p = 0.152$).

The frequency distribution of eyes with Matrix visual field alterations compatible with glaucoma associated with optic disc damage and/or HRT-3 assessed alterations was significantly higher in the AD group than in controls (27.5 vs. 7.5%; Chi-Square test; $p = 0.003$; OR = 4.69).

Remarkably, the study revealed that the mean IOP values of the two groups were in the normal range and, more interestingly, that AD patients had even lower IOP values than controls (ANOVA one-way test; $p = 0.000$). Moreover, the analysis of mean CCT values revealed no statistically significant difference between the groups (ANOVA One-way test; $p = 0.336$) (**Table 2**) ensuring an accurate measurement of the IOP.

Comparison of HRT-3 stereometric parameters between the groups showed significantly reduced RNFLt values (ANOVA One-way test; $p = 0.013$) in the AD group (**Table 2**). Contrastingly, no significant differences were found when the following stereometric parameters were considered: Rim area (ANOVA One-way test; $p = 0.271$); CSM (ANOVA One-way test; $p = 0.447$); HVC (ANOVA One-way test; $p = 0.141$); Cup/Disc Ratio asymmetry (CDRa) (Mann Whitney U test; $p = 0.399$) and vCDR (ANOVA One-way test; $p = 0.416$). An almost significantly difference was found when RIM Volume was considered (ANOVA one-way test; $p = 0.063$). In addition, the statistical analysis, as shown in **Table 2**, reveals a significantly worse MRA classification in the AD group (Mann Whitney U test; $p = 0.027$), but no significant differences in terms of GPS score (Mann Whitney U test; $p = 0.208$).

When the two global FDT perimetry indices were analysed, the mean MD values (ANOVA one-way test; $p = 0.000$) and mean PSD values (Mann Whitney U test; $p = 0.000$) resulted significantly higher in the AD group (**Table 2**). Finally, the GHT score was significantly higher in patients with AD than in controls (Mann Whitney U test; $p = 0.006$) (**Table 2**).

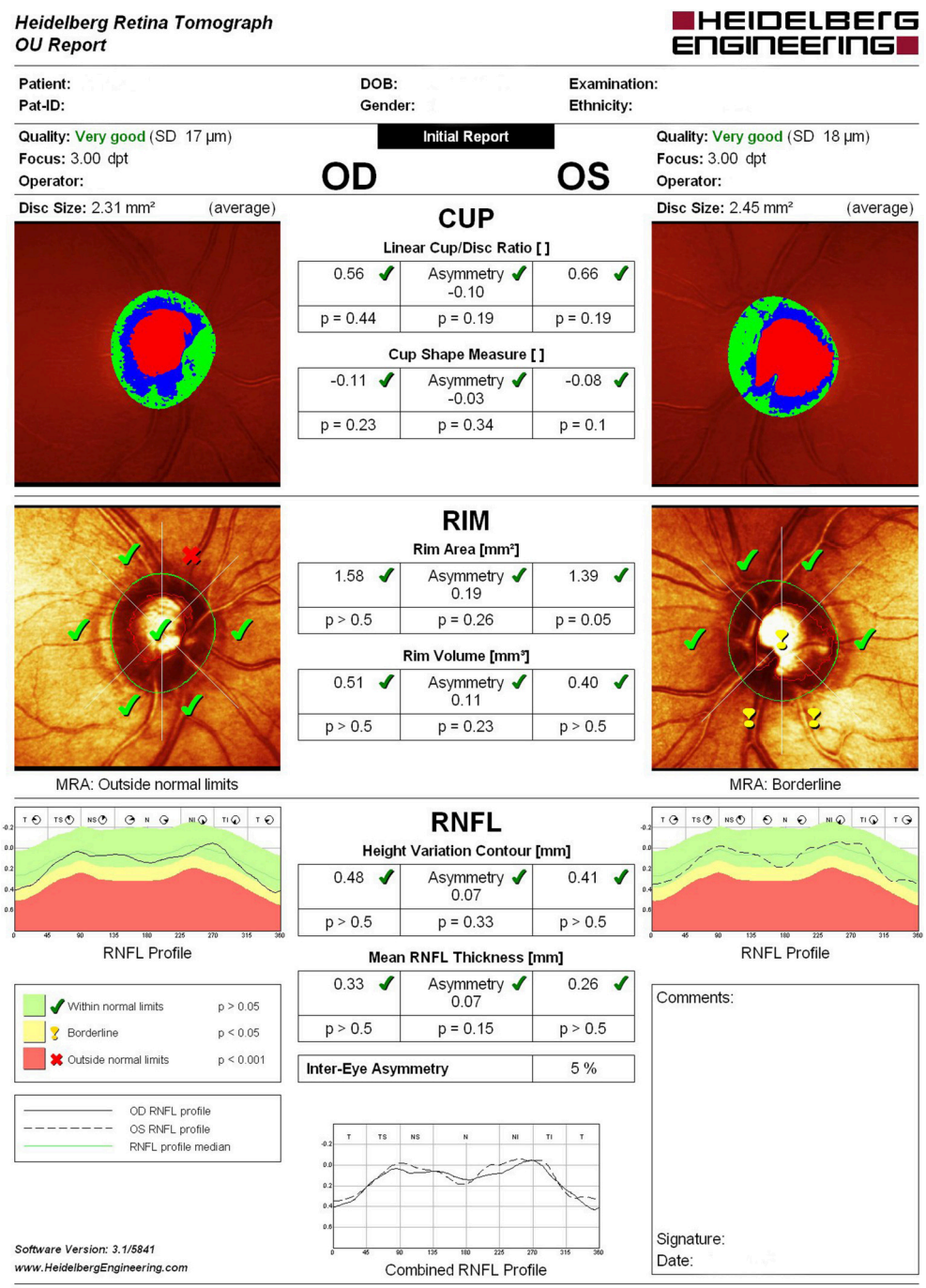


**FIGURE 1 | Heidelberg retinal tomograph III (HRT-3) analisys report.**

Correlations among MMSE and ocular parameters (MD, PSD, GHT, MRA, RNFL, IOP) were performed using Pearson's R correlation test. The test showed that Mini Mental State Examination is directly correlated with MD ($R = 0.349$; $p = 0.034$) (**Figure 2**) and inversely correlated with PSD ($R = -0.357$; $p = 0.030$) (**Figure 3**).

## DISCUSSION

Recent clinical studies have indicated a possible epidemiological link between glaucoma and AD, suggesting that patients suffering from the latter have an increased risk of developing glaucoma and tend to manifest the disease with a more severe clinical picture

**TABLE 1 | Descriptive analysis of the Alzheimer and control groups.**

| Group | Sex | Eyes | Mean age ± SD | Median | Min | Max | MMSE |
|---|---|---|---|---|---|---|---|
| CTRL | M | 29 | 69.9 ± 5.9 | 70 | 58 | 80 | – |
| | F | 38 | 68.2 ± 5.9 | 68 | 58 | 80 | |
| | Total | 67 | 68.9 ± 5.8 | 69 | 58 | 80 | |
| AD | M | 25 | 69.7 ± 6.6 | 69 | 58 | 80 | 20.8 ± 4.8 |
| | F | 26 | 71.4 ± 5.9 | 72 | 58 | 80 | 20.8 ± 3.6 |
| | Total | 51 | 70.6 ± 6.2 | 71 | 58 | 80 | |
| P | | 0.536[a] | 0.152[a] | | | | |

*AD, Alzheimer Group; CTRL, Control Group; MMSE, Mini Mental State Examination; SD, standard deviation; $p < 0.05$.*
*[a]ANOVA One-way test.*

**TABLE 2 | Statistical analysis of the ophthalmological parameters considered.**

| Variable | AD | CTRL | *P* |
|---|---|---|---|
| IOP | 13.31 ± 2.00 | 14.81 ± 2.25 | **0.000[a]** |
| CCT | 538.94 ± 35.34 | 544.84 ± 30.83 | 0.336[a] |
| RNFL | 0.17 ± 0.06 | 0.20 ± 0.58 | **0.013[a]** |
| Rim volume | 0.29 ± 0.12 | 0.33 ± 0.13 | 0.063[a] |
| Rim area | 1.35 ± 0.30 | 1.42 ± 0.35 | 0.271[a] |
| CSM | −0.17 ± 0.13 | −0.18 ± 0.06 | 0.447[a] |
| HVC | 0.30 ± 0.08 | 0.33 ± 0.07 | 0.141[a] |
| vCDR | 0.46 ± 0.19 | 0.43 ± 0.17 | 0.416[a] |
| CDRa | 0.07 (0.0; 0.13) | 0.05 (0.0; 0.32) | 0.339[b] |
| MRA | 1.6 (1.0; 3.0) | 1.3 (1.0; 3.0) | **0.027[b]** |
| GPS | 1.0 (1.0; 3.0) | 1.0 (1.0; 3.0) | 0.208[b] |
| MD | −6.19 ± 0.84 | −2.82 ± 0.49 | **0.000[a]** |
| PSD | 4.66 (1.91; 10.63) | 3.03 (0.18; 5.28) | **0.000[b]** |
| GHT | 4.0 (1.0: 4.0) | 2.0 (1.0; 4.0) | **0.006[b]** |

*Descriptives: Mean ± SD for gaussian variables and Median (min.; max.) for non-gaussian variables. IOP, Intraocular Pressure (mmHg); CCT, Central Corneal Thickness (μm); RNFL, Retinal Nerve Fiber Layer; CSM, Cup Shape Measure; HVC, Height Variation Contour; vCDR, Vertical cup/disc ratio; MRA, Moorfields Regression Analysis; GPS, Glaucoma Probability Score; MD, Mean Deviation; PSD, Pattern Standard Deviation; GHT, Glaucoma Hemifield Test; CDRa, Cup/Disc Ratio asymmetry; AD, Alzheimer Group; CTRL, Control Group; $p < 0.05$.*
*[a]ANOVA One-way test.*
*[b]Mann-Whitney U test.*

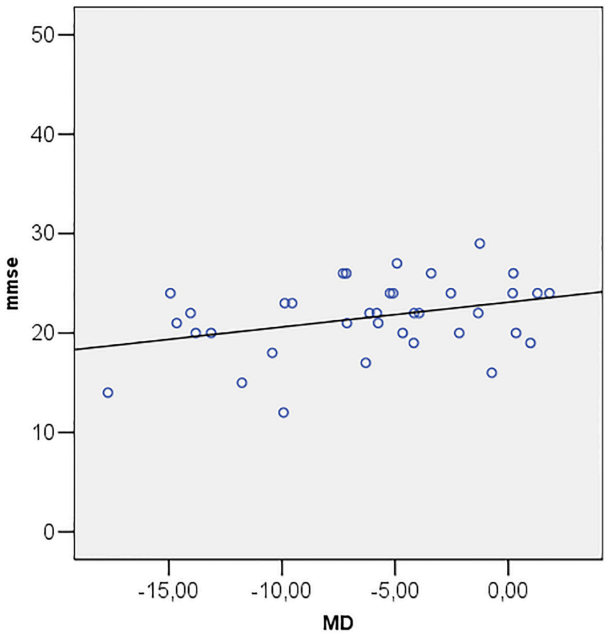


**FIGURE 2 | Scatter plot of mini mental state examination (mmse) vs. mean deviation (MD).**

(Bayer and Ferrari, 2002; Bayer et al., 2002a,b; Tamura et al., 2006; Lin et al., 2014; Pelletier et al., 2014). However, several studies have produced divergent results (Bach-Holm et al., 2012; Keenan et al., 2014). It is important to point out that several bias, such as diagnosis of POAG made by different clinicians using non-standardized diagnostic criteria, small sample size with wide confidence limits, affected some of these studies. Unlike previous reports, in this paper diagnosis of glaucoma was based on the occurrence of standardized diagnostic criteria, such as typical visual field defects and optic nerve changes and/or altered HRT-3 parameters.

Patients with cognitive impairment are less likely to produce reliable visual field test results than healthy subjects do (Trick et al., 1995). For this reason, previous studies, attempting to use the Standard Automated Perimetry (SAP) with Humphrey Field Analyser, have found that a high percentage of patients could not perform the visual field test. As a result, in those papers diagnosis of glaucoma relied only on ophthalmoscopic and stereometric examination of the optic disc. In this study the analysis of visual field parameters has been assessed by Matrix FDT, showing typical alterations of glaucoma, such as worse results in terms of MD, PSD, and GHT, in patients with AD. In addition, MD and PSD showed, respectively, a direct and an inverse correlation with MMSE in the presence of reliable visual field tests. Methodological justification for the use of Matrix FDT is grounded on its short test duration, on its reduced "learning effect" on test outcomes, and on Matrix

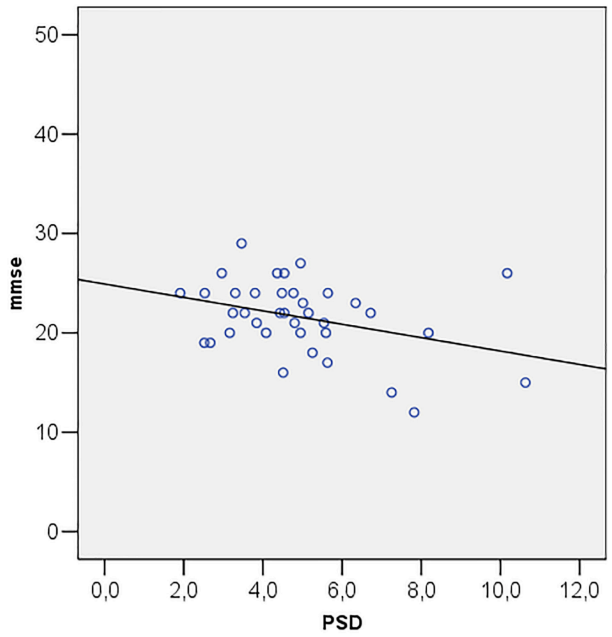


**FIGURE 3 | Scatter plot of mini mental state examination (mmse) vs. pattern standard deviation (PSD).**

FDT reported ability to identify glaucomatous visual field defects earlier than SAP (Cello et al., 2000; Pierre-Filho Pde et al., 2010). Overall, these features make the test more suitable in AD patients.

Furthermore, in contrast with previous reports (Kurna et al., 2014) morphological parameters of the optic nerve head and RNFLt, assessed by a standardized technique such as HRT-3, demonstrated statistically significant differences between AD and control group. In particular, in patients with AD, RNFLt was significantly reduced, and MRA was more frequently altered compared to controls. Many studies have demonstrated the ability of HRT to detect early structural alterations of the optic nerve, achieving results comparable to those obtained by glaucoma specialists (Deleón-Ortega et al., 2006). Interestingly, according to the literature, RNFLt is one of the stereometric parameters best fitting glaucomatous damage and its progression, showing high sensitivity and specificity (Uchida et al., 1996; Trick et al., 2006). Moreover, although MRA and GPS have demonstrated adequate specificity and sensitivity, some discs cannot be classified using GPS; thus endorsing a greater usefulness of MRA in glaucoma diagnosis (Andersson et al., 2011).

In the present paper, we have observed a 27.5% frequency of glaucoma-like alterations among AD patients, a value five times higher than in controls. These data strongly support the hypothesis that a significant percentage of patients with AD have a clinical picture similar to that found in glaucoma. Interestingly, we found a statistically significant difference in the mean IOP between the groups. Besides, AD group showed IOP values even lower than controls, thus supporting the hypothesis of an increased susceptibility to the IOP of optic nerve head of patients with AD. To ensure an accurate evaluation of the IOP, in contrast to previous studies that never carried out this test, we measured the CCT values. The analysis of CCT is of paramount importance because this is an independent risk factor for developing glaucoma and, even more, is a possible major source of overestimation (or underestimation) of the IOP value assessed by applanation tonometry. The finding that AD patients had CCT values not significantly different from those of the control population, as well as from the standard, ensure the absence of any bias in IOP measurement. This is important because, in our paper, a high proportion of AD patients presented clinical features similar to those found in glaucoma, with the exception of ocular hypertension. As a result, patients with Alzheimer's disease seem to present a reduction of the RNFLt and of the rim volume regardless of the ocular hypertension. It is therefore conceivable that this reduction could be the result of the extensive central neuronal loss, typical of Alzheimer's disease, independent of the IOP, which could affect the entire visual pathways. Consequently, in some cases, the clinical picture that we currently define as glaucoma may actually be the expression of a neurodegenerative disease of the central nervous system affecting, by transynaptic degeneration, the entire visual pathway altering the performance of important vision-related functions and quality of life (Nucci et al., 2013; Martucci et al., 2014; Cesareo et al., 2015). These results have been also confirmed by a recent meta-analysis, and other studies, that reported a significant RNFLt (He et al., 2012; Marziani et al., 2013) and macular volume reduction, assessed by optical coherence tomography (OCT), in AD when compared to healthy subjects (Gao et al., 2015). These data suggest a possible usefulness of the OCT in diagnosis of neurodegenerative disease such as AD (Larrosa et al., 2014; Rebolleda et al., 2015).

The mechanisms underlying the association between AD and glaucoma are the subject of intense debate in the literature. Interestingly Wostyn et al. (2009) reported a significant rate (25%) of very low cerebrospinal fluid pressure (CSFP) values in AD patients. Incidentally, this percentage is similar to that of glaucoma-like alterations found in this paper in AD patients. For anatomical reasons, IOP is counterbalanced by CSFP and optic nerve tissue pressure from the retro-laminar regions. It has been hypothesized that a reduction in CSFP can bring about a displacement of the lamina cribrosa, resulting in axonal damage at this point. Furthermore, clinical studies on NTG patients showed a significantly lower CSFP and a higher trans-lamina cribrosa pressure in these patients compared to healthy subjects. Therefore, the CSFP reduction may play a role in the optic nerve damage observed in patients with AD (Wostyn et al., 2009). This might be a possible mechanism explaining why, despite significantly lower IOP values, patients suffering from AD considered in this study had higher prevalence of glaucoma-like alterations than controls.

A second hypothesis, which may explain the link between the two diseases, is that the decrease in production and turnover of the CSF observed in patients with AD could reduce the clearance

of toxic substances in the subarachnoid space surrounding the optic nerve, thus activating neuroinflammatory processes (Killer et al., 2008; Ho et al., 2012). In this regard, it has been recently described the case of a glaucoma patient with medically controlled IOP who experienced a progression of the disease concomitantly with the onset of mild cognitive impairment. Interestingly, lumbar puncture revealed decreased Aβ, and elevated levels of total and phosphorylated tau (Nucci et al., 2011). It is therefore possible that deposits of tau and/or other toxic molecules also contributed to development and progression of glaucoma in patients with AD included in this study.

The cytotoxic effect of these substances has also been confirmed at cellular level by studies on autophagy (Hara et al., 2006; Levine and Kroemer, 2008; Jaeger and Wyss-Coray, 2010; Wong and Cuervo, 2010; Rodríguez-Muela and Boya, 2012). A reduction of Beclin-1, a gene product involved in the initiation and execution of autophagy, has been reported in AD patients. This seems to be associated with the accumulation of amyloid precursor protein and Aβ and, hence, neuronal cell death. In this regard, it has been recently observed that an acute rise of IOP, reducing Beclin-1, might derange the retinal autophagic machinery that constitutively occur in RGCs, causing their death (Russo et al., 2013). Therefore, all these mechanisms might have contributed to the morphological and functional damage detected by HRT and Matrix FDT.

## CONCLUSION

In conclusion, our data, collected using objective and standardized criteria, strongly support a link between AD and a higher risk of developing glaucoma-like alterations even without elevated IOP levels. Considering that epidemiological estimates are forecasting an exponential increase in Alzheimer's disease over the next 20 years, there is a risk that in the future we will face a large number of patients with optic nerve head and RNFLt alterations linked to this neurodegenerative disease.

## AUTHOR CONTRIBUTIONS

MC, AM, EC, RM, AC, AM, GS, CN: Substantial contributions to the conception or design of the work; the acquisition, analysis, and interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. RS: analysis and interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 19 April 2016
doi: 10.3389/fneur.2016.00055

# Visual and Ocular Manifestations of Alzheimer's Disease and Their Use as Biomarkers for Diagnosis and Progression

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Alzheimer's disease (AD) is the most common form of dementia affecting the growing aging population today, with prevalence expected to rise over the next 35 years. Clinically, patients exhibit a progressive decline in cognition, memory, and social functioning due to deposition of amyloid β (Aβ) protein and intracellular hyperphosphorylated tau protein. These pathological hallmarks of AD are measured either through neuroimaging, cerebrospinal fluid analysis, or diagnosed post-mortem. Importantly, neuropathological progression occurs in the eye as well as the brain, and multiple visual changes have been noted in both human and animal models of AD. The eye offers itself as a transparent medium to cerebral pathology and has thus potentiated the development of ocular biomarkers for AD. The use of non-invasive screening, such as retinal imaging and visual testing, may enable earlier diagnosis in the clinical setting, minimizing invasive and expensive investigations. It also potentially improves disease management and quality of life for AD patients, as an earlier diagnosis allows initiation of medication and treatment. In this review, we explore the evidence surrounding ocular changes in AD and consider the biomarkers currently in development for early diagnosis.

**Keywords: Alzheimer's disease, neurodegereration, biomarkers, animal models of neurodegenerative disease, visual changes**

OPEN ACCESS

***Edited by:***
*Raymond Chuen-Chung Chang, The University of Hong Kong, Hong Kong*

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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *06 January 2016*
***Accepted:*** *29 March 2016*
***Published:*** *19 April 2016*

***Citation:***
*Javaid FZ, Brenton J, Guo L and Cordeiro MF (2016) Visual and Ocular Manifestations of Alzheimer's Disease and Their Use as Biomarkers for Diagnosis and Progression. Front. Neurol. 7:55. doi: 10.3389/fneur.2016.00055*

## INTRODUCTION

The growth in life expectancy and the developing aging population has led to the increased prevalence of chronic diseases, such as Alzheimer's disease (AD). Globally, there are almost 46 million people in the world living with dementia, with the number expected to rise to 131.5 million by the year 2050 (1). According to the World Health Organization (WHO) Global Burden of Disease (2004), dementia is the second largest contributor leading to total number of years living with disability (YLD) in people aged 60 years or older at 13.5%, compared to heart disease (4.0%), stroke (4.4%), and cancer (2.2%) (1).

Pathologically, AD is characterized by deposition of extracellular senile plaques, which is composed of amyloid β (Aβ) and intraneuronal neurofibrillary tangles (NFTs), resulting from intracellular aggregates of hyperphosphorylated tau protein detected in the brain (2). Aβ plaque deposition is associated with cross-sectional synaptic network dysfunction, progressive brain atrophy, and longitudinal cognitive decline (3). Studies have shown that Aβ and tau pathology correlates

with neurocognition in mild cognitive impairment (MCI) (4). Less specific neuropathological lesions include granulovacuolar degeneration and eosinophilic rod-like bodies known as Hirano bodies (5).

Presently, AD can only be diagnosed post-mortem on histopathological examination. Diagnostic investigations are limited, and physicians rely on clinical examination and exclusion of differential diagnoses that may cause cognitive impairment, such as depression, Parkinson's disease (PD), hypothyroidism, drug interactions, and vitamin deficiencies (6). Clinically, a diagnosis is made based on history, examination, and where available, accounts from relatives or carers. Nevertheless, premortem diagnoses have been considered inaccurate in 10–15% of cases even when assessed by experienced clinicians (7). Given the difficulties and delay in clinical diagnosis, patients often develop pathological damage prior to starting treatment. The advance of biomarkers using magnetic resonance imaging (MRI), positron emission tomography (PET), and cerebrospinal fluid (CSF) have led to the development of guidelines and diagnostic criteria (8–10). SPECT scanning can also be used to detect regional reduction in cerebral blood flow thought to be present in patients with AD (11).

Alzheimer's disease is a heterogeneous disease and has multiple cognitive subtypes. These are usually broken down into memory, language, executive, attention, and visuospatial functioning (12). The variant of AD in which visual symptoms are prominent due to the localized pathology in the parieto-occipital region is often referred to as visual variant Alzheimer's disease (VVAD) (13).

The interconnection between eye and brain suggests that it is reasonable to look for ocular manifestations of neurodegenerative disease and regard the eye as an extension of the CNS.

In embryological development, the eyes and brain have a similar origin. The eyes are formed from the anterior neural tube, an area that later gives rise to the forebrain. Ocular development occurs through specification of the eye field post-neural induction (14). This process involves specific transcription factors that are also conserved in brain development. One such factor, a "master regulator" gene of the development of the eye field, Pax6, plays an essential role in neural development. When expressed ectopically, Pax6 can induce ocular formation in other parts of the body (15), whereas its impairment or knockout disrupts neurogenesis in the cortex (16, 17).

In the eye, retinal neurons are comparable in many ways to their counterparts in the brain. Retinal neurons have dendrites, a soma and an axon, which are the essential neuronal features (18). They are stained by many typical neuronal markers (19–21). They form complex information processing networks similar to those in the cerebrum (22–24). The retina contains over 60 types of neuron (25) that play distinct roles in information processing. Photoreceptors represent one of the five main types of cells, the others being horizontal, bipolar, amacrine, and ganglion cell types. Photoreceptors transmit signals in a neuronal fashion when excited by light and are separated into two main subtypes, rods and cones. Bipolar cells transmit this information to retinal ganglion cells (RGCs). Horizontal cells are also connected to photoreceptors as well as bipolar cells and provide inhibitory feedback, to both adjust and refine the light signal. Amacrine cells can modify direct signals between bipolar and ganglion cells or can act as intermediaries between the two (26). In retinal networks, amacrine, bipolar, and horizontal cells act similar to interneurons, and the RGCs behave as projection neurons. Similar classes of neurotransmitters have been found in the retina, such as GABA and glutamate, which are essential to retinal information processing (27–29). Recently discovered group of retinal cell subtype are the intrinsically photosensitive retinal ganglion cells (ipRGCs). These cells respond to light through expression of the photopigment melanopsin in the absence of rod and cone photoreceptor input (30).

Therefore, due to its close association with the brain, it is not surprising that neurodegeneration caused by disorders such as Alzheimer's extends into the eye. Visual symptoms have been well documented in AD, and there is significant evidence to illustrate that ocular pathology occurs as part of the disorder (31, 32). Consequently, this provides an opportunity to use a minimally invasive approach to examine the pathological features in the brain – through the transparent medium of the eye.

A critical component of research into AD is the use of animal models to standardize and replicate features of the disease in order to develop and assess response to treatments that would otherwise be unethical in human subjects. Approximately 98% of potential drug therapies fail in Phase 3 clinical trials, thus, pushing the focus of research toward targeting molecular pathways rather than symptom control (33). Animal models need to fit three validation criteria when used for human research (34). These are face validity, predictive validity, and constructive validity, which aim to ensure that animal model is based on relevant interpretation of the human condition.

With this in mind, many animal models have been proposed, including dog, rhesus monkey, *Drosophila melanogaster*, rat, and mouse (35). In particular, the mouse model is the most popular, as it has been noted to be most similar to human CNS structure in addition to its relatively inexpensive production of multiple transgenic strains (36). Transgenic mouse models of AD express single, double, or triple mutations found in familial AD. Genes encoding these mutations are presenelin 1 (PS1), presenelin 2 (PS2), tau, and amyloid precursor protein (APP). It is not the scope of this review article to detail all transgenic models available, but for the reader to have a brief background into the mutations involved.

There is a growing interest to identify a biomarker for AD to enable early diagnosis and prevent cognitive deterioration in patients. Genetic testing in AD has identified three genetic loci, *APP*, presenilin-1 (*PSEN1*), and presenilin-2 (*PSEN2*) as susceptibility genes for early-onset AD (EOAD) and SORL1 and APOE for late-onset AD (LOAD). Although these genes are useful in predicting the risk of developing AD, their lack of diagnostic specificity and sensitivity and the influence of external environmental factors make them unsuitable as biomarkers for AD (37–39). The majority of research has focused on the retina and associated changes in thickness, inflammation, and cell death. Nevertheless, manifestations of AD have additionally been identified in the pupil, lens, choroid, and optic nerve, though their use in the clinical setting has not been established (**Table 1**). Furthermore, changes in visual function have been identified in multiple studies (**Table 2**) and are also being investigated as potential indicators of AD pathology.

**TABLE 1 | Manifestations of Alzheimer's disease (AD) in the eye.**

| Orbital structure | Pathological changes in AD |
|---|---|
| Pupil | Atypical pupil response to cholinergic antagonists (53, 54)<br>Lower amplitude and latency of maximum reaction of pupillary light reflex (59)<br>Increased pupillary size (62) |
| Lens | Aβ in lens and aqueous humor (66)<br>Predisposition to supranuclear cataract (66) |
| Retina | Decreased retinal blood flow and RNFL thinning (75, 82, 83)<br>RGC degeneration particularly in superior and inferior peripheral retina (100, 106)<br>Overall reduction in RGC axon numbers (26)<br>Aβ deposition in retina (94) |
| Choroid | Reduced choroidal thickness (101) |
| Optic nerve | Increased cup: disk ratio and pallor (148–150) |

**TABLE 2 | Visual manifestations of AD.**

| Indicator of vision | Manifestation of AD | Recommended clinical test[a] |
|---|---|---|
| Visual acuity | Decreased visual acuity in low luminance (32, 33) | HOTV chart |
| Contrast sensitivity | Reduced visual contrast sensitivity particularly in low frequencies (37, 38)<br>Reduced reading speed at lower contrast sensitivities (37) | Pelli–Robson chart (35)<br>Michelson contrast test (37) |
| Color vision | Poor color discrimination (43)<br>Deficiencies most significant in tritan axis (31, 42) | City University test<br>Ishihara test (154) |
| Visual field loss | Inferior hemifield loss (45) | Humphrey automated perimetry (45)<br>FDT (46) |
| Motion perception | Higher thresholds for motion detection across all spatial frequencies (57) | Computer animation sequences using random dot cinematogram (57) |
| Depth perception and stereopsis | Reduced stereopsis, mean threshold >150 s of arc | Randot stereotest (155) |
| Ocular motor function | Abnormal hypometric saccades<br>Increased latency as compared to controls (50, 51) | Eye movement examination (62) |

[a]*All clinical tests require patient cooperation, which can be difficult in AD patients.*
*AD, Alzheimer's disease; FDT, frequency doubling technique.*

# VISUAL MANIFESTATIONS OF AD

## Visual Acuity

Many studies have found no significant difference in visual acuity between AD patients and control subjects (40). Levels of luminance may affect visual acuity, as demonstrated in AD patients who had decreased visual acuity under conditions of low luminance (LL) (41, 42). A recent study has shown that AD patients have a significantly poorer accuracy and ability in recognition of pictures in LL and low spatial frequency (LSF), compared to age-matched controls (43). Another important factor to note in AD patients is the increased prevalence of cataract affecting visual acuity, which is addressed later in this review (44).

## Contrast Sensitivity

Contrast sensitivity allows for the ability to recognize objects over a range of spatial frequencies and is usually tested using charts (45) or electronic equipment. A deficit in this visual function greatly affects the daily functioning and quality of life and may explain the increased risk of falls and fractures in AD patients (46). AD patients have markedly reduced visual contrast sensitivity as compared to age-matched controls (47, 48) and are sensitive to testing even in early stages of AD. Furthermore, donepezil, an anticholinesterase inhibitor, has been shown to improve contrast sensitivity in AD patients (49). A reduction in reading speed has also been noted, particularly at lower contrast sensitivities (47). Studies have also shown that reading latency is increased in AD patients and is more evident with irregular words in text (50).

## Color Vision

Köllner first described changes in color vision, according to area of the eye affected, correlating retinal disease with blue–yellow visual changes and optic nerve disease with red–green visual loss (51). Earlier studies in AD patients have indicated that there are deficiencies in the tritan (blue) axis (40, 52), but more recent studies show no significant interaction in color axes (53). However, they do report an inversely proportional correlation with mini-mental state examination (MMSE) score and color discrimination error.

## Visual Field Loss

Visual field (VF) loss in AD is likely attributed to the neurodegenerative changes and synaptic dysfunction, particularly due to Aβ accumulation (54). Humphrey automated perimetry has been used in VF testing between AD patients and age-matched controls and has shown that the sensitivity losses occur particularly in the inferior hemifield of AD patients, and furthermore that degree of loss correlated with degree of dementia (55). VF testing with frequency doubling technology (FDT) also found that AD patients had VF deficits as compared to age-matched controls (56).

## Motion Perception

Motion perception is the process of deducing speed and direction of elements and is vital to everyday functioning and navigation. Studies have detected higher thresholds for motion detection in AD patients as compared to age-matched controls, and further correlation with dementia severity (57). Another recent study looked at visual motion processing and again found that patients with AD had higher thresholds across all spatial and temporal frequencies (58).

## Depth Perception and Stereopsis

Stereopsis is the perception of depth and 3D structure obtained from binocular vision. Studies have shown that patients with AD have reduced stereopsis as compared to control groups, and some

also note a correlation between cognitive assessment scores and performance on stereopsis testing (59, 60).

### Ocular Motor Function

Alzheimer's disease patients are known to have ocular motility dysfunctions and poor visual attention. They are often unable to focus on a fixed object, due to their difficulty in suppressing reflexive saccades. Crawford et al. used "eye tracking" software to assess saccadic eye movements in AD patients and found that they had slower reaction times as compared to the control group (61). Other studies have also shown patients with AD presented with abnormal hypometric saccades when tested, in addition to increased latency as compared to controls (62, 63). In particular, anti-saccades require movement of the eye in the opposite direction of a visual stimulus and suppression of the reflexive response. Functional MRI (fMRI) studies have shown patients with AD exhibited significantly more anti-saccade errors and, overall, demonstrated reduced activity in all oculomotor regions of the brain as compared to controls (64). The convergence angle has also been noted to be smaller and more irregular in AD patients (65).

## PUPIL

It is now well established that patients with AD have an acetylcholine deficit and an altered ACh pathway (66). This became apparent in 1994 as a hypersensitive pupil response to cholinergic antagonist, tropicamide (0.01%), was discovered (67). AD patients' pupils dilated to 13% more than controls. Subsequent experiments by Iijima et al. (68) found that significant differences in pupil sizes were found at a lower dose of tropicamide (0.005%), potentiating the possibility for a diagnostic screening test. Scinto (69) further investigated pupil involvement in AD and located a clear biological link between ApoE allele status and pupillary response to dilute tropicamide. Nevertheless, the use of the tropicamide test as a diagnostic tool for AD is controversial, as other studies have failed to find significant results (70–72).

Another indicator of AD pathology is the pupillary light reflex (PLR). Changes in the oculomotor system of the Edinger–Westphal nucleus and degeneration in the nucleus basalis of Meynert leading to cholinergic deficit are likely to lead to changes in the pupillary system. Pupillometry using image analysis technology can provide multiple parameters from a single PLR test, such as latency to pupil reaction, constriction acceleration and velocity, and amplitude of response. Prettyman et al. first described 75% latency of normal pupil size and the differences in constriction amplitude between AD patients and controls (73). Repetitive stimulation of the pupil light reflex over time is less pronounced in AD patients as compared to controls, with lower amplitude and latency of maximum reaction (74). Certain PLR measures have been found to correlate with cerebral plaque burden (75). Moreover, Bittner et al. found a significant correlation between increased pupillary size and CSF measures of both Aβ and tau (76). Color pupillometry has been used to measure the response of ipRGCs in various eye disorders (77, 78), but not yet tested in AD patients. This may be a potential biomarker requiring further investigation in future studies.

## LENS

Interest in the lens as an indicator of AD began after APP and Aβ expression were discovered in cultured mammalian lenses (79). In animal models, Aβ transgenic mice have developed lens opacification and shown to improve with EUK-189, a synthetic SOD/catalase, demonstrating a link between AD-associated cataracts and oxidative stress (80). Dutescu et al. also noted significant Aβ deposition in the lens of transgenic mice when labeling with WO2 and 1E8 antibodies, consistent with results from human studies (81).

In humans, Goldstein et al. detected the presence of Aβ in the lens and aqueous humor of AD patients (82). They also found that AD patients had a specific cataract in the supranuclear region not present in controls, which had high reactivity and staining against Aβ markers and antibodies. Similar cataracts have been found in Down syndrome patients, who have an increased level of Aβ due to a triplication of the APP gene (83). However, Michael et al. (84, 85) and Ho et al. (86) found no staining of Aβ in the lens of AD patients, although using different staining and characterization methodologies (84–86). Moreover, in a recent study, Bei et al. have concluded that opacities of the lens cannot be used as a non-invasive risk marker, as it does not vary significantly when controlling for age (87). Nonetheless, a clinical study by Kerbage et al. found that a fluorescent ligand for Aβ could be used *in vivo* to differentiate between AD patients and controls (88). There was a twofold greater signal in the AD patients compared to controls.

A subsequent clinical trial found the fluorescent signal of the same ligand to be able to distinguish between clinically diagnosed AD patients and controls with better results than a PET marker (89). This is a promising new development for diagnosing Alzheimer's, although few studies have investigated changes during early stages of the disease. The incidence of cataracts and AD increases with age. This is important to note when using the lens as a biomarker for AD, as many patients will develop cataract irrespective of AD and, furthermore, may have undergone cataract surgery, thus limiting the use of the lens as a biomarker.

## RETINA

### Retinal Vasculature

Similar to the brain, the retina has a highly isolated and thoroughly protected vasculature (90). Since there are vascular changes in the AD brain (91), it is likely that the analogous changes may be found in the AD retina. In a preliminary study, Berisha et al. found decreased vein diameter and decreased blood flow (92). In two recent large-scale studies ($n = 456$), AD patients had a less complex venular structure, smaller, more sparse, and tortuous retinal vessels (93, 94). Furthermore, recent studies have found significantly decreased retinal venous blood flow in AD patients (95). Importantly, MCI patients' blood flow was found to be intermediate between AD patients and controls. This suggests that retinal blood flow (microliter per minute) might be used to monitor disease progression. They also observed that retinal nerve fiber layer

(RNFL) thickness was decreased, but not significantly between AD patients and both MCI and control groups. This suggests that blood flow changes may precede cell death in the retina. Retinal oximetry is used to detect changes in eye metabolism and has been noted in a recent study to show abnormalities in AD. Einarsdottir et al. found that retinal oxygen saturation in arterioles and venules was elevated in AD patients as compared to controls (96).

## Retinal Thickness

Early histopathological studies implicated RGCs as the primary targets of cell loss in AD (97). As they demonstrated that the outer layers were relatively preserved in the postmortem retinas of AD patients, measurements have predominantly focused on the RNFL. Imaging technology, such as optical coherence tomography (OCT), has recently illustrated further evidence of RGC degeneration *in vivo* through measurement of the RNFL, indirectly evidencing ganglion cell loss (92, 98–100). An initial study by Parisi et al. found decreased RNFL thickness in all four retinal quadrants (101). RNFL thinning correlated with patients' pattern electroretinogram performance, suggesting that RNFL thinning is related to visual dysfunction in AD. Current evidence also suggests that RNFL thickness decreases as the disease progresses and that there is a significant correlation between overall macula volume and level of cognitive impairment measured by the MMSE (102). Studies have shown significant differences not only between MCI patients and controls but also between MCI patient's and two other AD groups (moderate and severe AD) in superior and total RNFL thickness (103). A recent meta-analysis (104) of 11 AD OCT studies found significant reduction in mean RNFL and in all 4 individual retinal quadrants around the AD macula. In three of these studies containing MCI patients, decreased RNFL thickness was found compared to controls. Bambo et al. also noted significant RNFL thinning in the superior and inferior quadrants of AD patients (105).

A few animal studies have published similar findings with regards to neuronal cell loss in the RGCL. One study using a single transgenic mouse model reported a statistically significant decrease in the retinal thickness of the Tg2576 mice from the GCL to the ONL as compared to wild-type control animals (106). This suggests that retinal degeneration may affect all retinal cell types and layers.

Recently, Ong et al. (107) combined the use of OCT and MRI scanning in an elderly population (>60 years old). They discovered that decreases in GC-IPL thickness correlated with decreased size of the occipital and temporal regions of the brain. These correlations suggest that degeneration in the retina is paralleled in specific regions of the brain, which are implicated in AD.

## Aβ in the Retina

Synaptic dysfunction and neuronal cell death because of Aβ toxic deposits are pathological hallmarks of AD (54). In animal models, overexpression of the human APP Swedish gene in Tg2576 mouse results in deposition of Aβ plaques in the brain (81). This strain of mice developed memory deficits at 10 months of age as compared to the APPswe/PS1-del9 transgenic strain that developed deficits earlier at 6 months of age (108). Aβ plaques have since been reported in the same strain, from the ganglion cell layer to the inner plexiform layer, in addition to plaques found in the outer segment and optic nerve (106, 109) (**Table 3**). Thus, this model suggests that ganglion cells and potentially retinal interneurons (horizontal, bipolar, and amacrine cells) are affected by Aβ plaques; yet, all retinal cells may be compromised in later stages of the disorder.

**TABLE 3 | Location of APP and Aβ found in the animal retina.**

| Retinal layer | APP | Aβ | Tau |
|---|---|---|---|
| Retinal pigment epithelium | – | – | – |
| Outer nuclear layer | – | + | – |
| Inner nuclear layer | + | + | – |
| Inner plexiform layer | + | + | – |
| Ganglion cell layer | + | + | + |

*+Present.*

*–Absent.*

*APP has been found in the ganglion cell layer through the inner nuclear layer in AD animal models (65, 81, 86, 106). Aβ has been found in the retina in all layers apart from the retinal pigment epithelium (81, 88, 93, 109, 114). Tau has been found in ganglion cell layer (99, 106, 133).*

Amyloid β loads are further associated with immunoreactivity for MCP-1, F4/80, and TUNEL-positive profiles in the RGC layer in mutant presenilin (PS1) and APP transgenic mice. This further indicates that Aβ deposition causes retinal neurodegeneration in mouse models of AD (110). Transgenic mice overexpressing the Swedish mutation (APP23) and mutant human APP and mutant human presenilin-1 (APPPS1) exhibit a threefold increase in total endogenous murine Tau in CSF and an age-related increase in Aβ deposition (111).

*In vivo* imaging in APP/PS1 transgenic mice following administration of systemic curcumin has provided a potential tool for monitoring Aβ plaque formation in AD (112). Optical imaging showed increased plaque formation with age in the mouse model, and remarkably, a decrease in response to glatiramer acetate immunotherapy. APP and Aβ have both been located in the human retina (113); however, these were not consistently found (32). A major advance was made in 2011 in human AD, where Aβ was visualized in the postmortem retina and also live animal retina (114). Very recent work has revealed substantial Aβ deposition in postmortem AD retinas and that these deposits may preferentially target the melanopsin-staining subtype of RGC (115).

Amyloid β has been identified in retinal drusen, a hallmark of age-related macular degeneration (AMD), a major cause of worldwide blindness. Drusen are abnormal extracellular deposits along the basal surface of the retinal pigmented epithelium (RPE). Aβ-containing drusen are associated with RPE atrophy and photoreceptor death (116, 117). In AMD, they are predominantly located in the macula at the center of the retina, but they can still occur peripherally. In fact, peripheral drusen have been found to be significantly associated with AD (118, 119).

It is believed that Aβ can enter the RPE through advanced glycation end products (RAGE)/p38 MAPK-mediated endocytosis. Intracellular Aβ triggers breakdown of RPE tight junctions (120), as described in Tg AD mice (121). The toxic effects of Aβ on RPE include reduced mitochondrial redox potential and increased

reactive oxygen species (ROS) production, RPE pigmentation and hypertrophy, followed by photoreceptor death (122). In addition, Aβ-mediated gliosis of Müller cells (MCs) has been implicated in retinal degeneration. MCs are the principal retinal glial cells and metabolically coupled to photoreceptors. Glial cell activation in response to Aβ deposition has compromised the integrity of the blood–retina barrier, leading to photoreceptor apoptosis (123). Interestingly, Aβ did not induce cell death in purified photoreceptor cell cultures but in mixed retinal cell cultures, suggesting that the cellular environment plays a role in Aβ-mediated photoreceptor apoptosis (124). Furthermore, Aβ has been implicated in complement activation by upregulating factor B, the main activator of the complement alternative pathway, in RPE through cytokines, which are released from recruited macrophages/microglia (125). This may explain the colocalization of Aβ with activated complement components found in some drusen (126, 127). Similar observations have also been reported in senile Aβ plaques in the brain, where Aβ is thought to be a primary activator of complement in AD (128), suggesting common mechanisms may be applied to AD, drusen formation, and AMD.

### Tau in the Retina

Tau accumulation has been observed in brains of doubly transgenic mice from 4.5 months of age (129) and in the hippocampus and amygdala of triple-transgenic mice (130). In single Tg2576 transgenic mice (APP), hyperphosphorylated tau was observed in adjacent sections of Aβ deposition in the ganglion cell layer (106). Double-transgenic mice APP/PS1 also displayed hyperexpression of tau in the retina with consequent upregulation of p35, p25, and calpain, which has been widely hypothesized to cause synaptic dysfunction and calcium dysregulation in the context AD-related apoptosis (131, 132). In human P301S tau transgenic mice, tau aggregates formed in the RNFL resulting in axonopathy and at 2 months of age formed tau inclusions within RGC (133). Tau has been found in the human retina (113); yet, this finding has not been consistently replicated (86, 134).

### Retinal Fluorescence and Neurodegeneration

In animal models, retinal changes observed in double-transgenic mice included accumulation of Aβ peptides in addition to detection of apoptotic cells in the RGC layer (135). Another study using the double-transgenic model (Tg2576 × Tg1) observed that 27-month-old mice had a 200% increase in the number of apoptosing cells in the GCL, as compared to 7.8-month-old mice (110).

A more extensive triple-transgenic mouse model expressing human APP, PS1, and tau mutations was used to investigate retinal glial changes in AD. The study by Edwards et al. found that at 9 months, MCs were activated, and astrocytes increased in size and number indicating retinal glial activation (136). Further evidence for involvement of the outer retina in AD models of disease is depicted in transgenic AD rat models. Tsai et al. found on staining of retinal sections that RPE cells showed marked hypertrophy and frequently contained two nuclei rather than one (137).

*In vivo* imaging has recently developed to allow direct visualization of apoptosing cells in the retina. A novel technique has been established using radiolabeled annexin V and confocal laser scanning ophthalmoscopy to detect cell apoptosis in the retina (138). This *in vivo* imaging method is known as detection of apoptosing retinal cells (DARC). Using this imaging technique and applying it to animal models provides further evidence of RGC apoptosis in AD. Following PI and annexin-IR intravitreal injection in triple-transgenic AD mice, a significant number of RGCs were observed to undergo early-phase apoptosis as compared to age-matched controls (139).

A small pilot study using retinal fluorescence lifetime imaging ophthalmoscopy (FLIO) suggested that retinal changes detected by FLIO correlated with clinical characteristics of AD and could potentially be used as a biomarker for AD. However, the lack of control group and small study size warrants further research before attributing these general changes specifically to AD (140). Another study used systemic curcumin administration followed by optical imaging to successfully label retinal Aβ plaques in mice (114). This has also been trialed in patients using curcumin supplements and retinal fluorescence imaging (141). Furthermore, recent research has established methods of identifying drusen deposits in the peripheral retina using ultra-wide-field imaging (142, 143). This is particularly of note in patients with AD, as these small deposits were not found in age-matched controls, thus providing possibility for another potential biomarker for diagnosis of early AD.

## CHOROID

Most research has been directed toward retinal changes in AD given the direct association with the brain. However, a few studies have observed possible choroidal manifestations of AD in animal models. Heterozygous transgenic rats (TgF344-AD) had significantly reduced choroidal thickness as compared to age-matched controls (137). Also, of note in this particular study was the upregulation of complement factor C3, which has previously been noted to play a role in RGC related apoptosis, studied particularly in glaucoma (144). Ning et al. also reported age-dependent Aβ deposits in the retinal and choroidal vasculature of two strains of transgenic mice (110).

In human models, recent imaging of the choroid in AD patients showed decreased choroidal thickness (145). However, given the limited research undertaken, more studies are needed to confirm if the choroid is indeed damaged in AD.

## OPTIC NERVE AND NEURODEGENERATION

Cell death of RGC and changes in the optic nerve head have long been noted in postmortem AD retinas and first described by Hinton et al. who found a variety of degenerative profiles in RGCs, including cell shrinkage and cell swelling with vacuolization (97). Moreover, there was a two to threefold reduction in RGC axon numbers compared to controls. Further research (146) found the same features of degeneration in addition to

nuclear fragmentation and heavily silver-stained cytoplasm. It was also noted that AD optic nerves had approximately half the RGC axon density of controls (32). This suggests that the larger "M" cell type RGCs are chiefly affected by AD. Blanks et al. found a 25% reduction of RGCs in the central 3 mm of the AD retina and severe cell loss over the entire retina (134, 147). This degeneration is more pronounced in the superior and inferior peripheral regions.

More recently, new imaging technologies have provided *in vivo* evidence of optic nerve head pathology. Multiple studies, using OCT and confocal laser scanning, have found greater cup-to-disk ratio and increased pallor of the AD optic nerve, representing a significant loss of RGC axons (148–151).

## FUTURE IMPLICATIONS

As outlined earlier in this review article, specialist brain imaging modalities, such as fMRI and PET scanning, can support a diagnosis of AD. Unfortunately, these are expensive investigative techniques and are often limited to a hospital setting. Furthermore, changes in the brain are often a sign of later-stage AD and symptoms of cognitive decline will already have set in. Remarkably, studies now support the hypothesis that Aβ plaques appear earlier in the retina (114) and so propose the possibility for earlier diagnosis of AD and subsequent treatment.

Alzheimer's disease-related retinal degeneration has provided a novel technique for investigating AD pathology and targeting treatment. Multiple human and animal studies have illustrated the correlation of AD neuropathology in the retina and the brain, including Aβ and tau accumulation, neuronal cell loss, and RGC apoptosis. Using the eye and its transparent medium as an "extension of the brain" allows for non-invasive visualization of AD pathology *in vivo*, and enables monitoring of biomarkers in order to diagnose and potentially monitor development and treatment of AD.

Technology, such as DARC, has provided a platform for this (139), and different approaches to *in vivo* imaging are constantly evolving (114, 143, 152). Unfortunately, these methods of *in vivo* imaging are not without limitations, particularly with regards to specificity for Aβ plaques. Currently, the use of OCT to assess the ONH and RNFL are useful in detecting neurodegeneration, in addition to a comprehensive eye examination in the clinical setting covering VF defects, pupillometry, and contrast sensitivity. *In vivo* imaging is emerging as a potential biomarker in many neurodegenerative diseases (138, 153), and the evolution and development of such imaging techniques today prove promising as diagnostic tools of the future.

## AUTHOR CONTRIBUTIONS

FJ: preparation of manuscript, editing, proofing and submitting author; JB: preparation of manuscript, editing; LG: editing manuscript; MC: overall editing and corresponding author.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

OPINION
published: 27 July 2016
doi: 10.3389/fneur.2016.00119

# Links between the Brain and Retina: The Effects of Cigarette Smoking-Induced Age-Related Changes in Alzheimer's Disease and Macular Degeneration

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**Keywords: Alzheimer-like pathology, age-related macular degeneration, cigarette smoking, retina, amyloid peptide**

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***Edited by:***
*Wendy Noble,*
*King's College London, UK*

***Reviewed by:***
*Mark P. Burns,*
*Georgetown University, USA*

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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *10 November 2015*
***Accepted:*** *08 July 2016*
***Published:*** *27 July 2016*

***Citation:***
*Yu SS, Tang X, Ho Y-S, Chang RC-C and Chiu K (2016) Links between the Brain and Retina: The Effects of Cigarette Smoking-Induced Age-Related Changes in Alzheimer's Disease and Macular Degeneration. Front. Neurol. 7:119. doi: 10.3389/fneur.2016.00119*

Alzheimer's disease (AD) is characterized by the progressive and selective loss of neurons and synapses. This debilitating disease was estimated to affect 33.9 million patients worldwide in 2011, a number that is expected to triple over the next 40 years (1). It has been shown that a combination of several processes, including extracellular deposition of amyloid-beta (Aβ) plaques and the formation of intracellular neurofibrillary tangles (NFTs) composed of hyper-phosphorylated tau proteins, in the brain are involved in the declining cognitive processes associated with AD (2). While dysfunction of the aforementioned biological processes starts from Braak stage I (preclinical AD), it is not until the majority of the neocortex is severely affected by neurofibrillary changes (Braak stages V–VI) that patients are ultimately diagnosed with dementia (3).

Notably, clinical studies have found numerous links between AD and age-related macular degeneration (AMD). AMD is a progressive macular neurodegenerative disease and is the most common cause of irreversible blindness, being estimated to affect 196 million people globally by the year 2020 (4). Although AMD is largely considered a retinal disease, the emerging epidemiological links between cognitive impairment, such as those observed in AD patients, and AMD patients are significant (5). In fact, it appears that cognitive impairment and early AMD may share numerous common age-related pathways and risk factors (6–8). Using logistic regression models that control for age, sex, race, education, systolic blood pressure, total cholesterol level, diabetes mellitus, smoking status, and ApoE genotype, it has been demonstrated that persons with mild cognitive impairment were more likely to have AMD compared to healthy patients (odds ratio of 2.00). It also appears that the reverse is true, with the prevalence of mild cognitive impairment being higher in AMD patients compared to control groups (52.4 vs. 26.8%, $p < 0.001$), with an odds ratio of 3.127 after adjusting for age, education, and visual acuity (8).

The similarities between AD and AMD are not that surprising as the retina is an integral part of the central nervous system (CNS), being derived from the neural tube much like other regions of the brain. AMD and AD also have many parallel characteristics, which have been extensively reviewed by Ohno-Matsui (9). One of the common pathological hallmarks of these two diseases is the extracellular deposits that are highly enriched with Aβ42. In AMD, the deposits, called drusen, are found between the retinal pigmented epithelium (RPE) and Bruch's membrane. In AD, the

deposits found in the brain are referred to as senile plaques and are localized primarily to the hippocampus and cortex. Detailed proteomic analyses of their molecular components have shown similarities between drusen and senile plaques, and proteins such as Aβ, tau, proteoglycans, and inflammatory mediators have been detected in both. These findings suggest that similar pathways could potentially be involved in the etiology of both AMD and AD.

Aβ42 peptides and related amyloidogenic molecules are thought to impact AMD and AD pathology in the following ways: (i) through progressive distortion of the structure and function of the retinal/neocortical architecture; (ii) by promoting mitochondrial dysfunction and the generation of reactive oxygen species (ROS) leading to the oxidation of retinal/neuronal components and apoptosis; (iii) by activating microglial cells, the chief innate-immune "phagocytosis" and "scavenging" cells of the retina/brain and CNS; (iv) by activating microglial-mediated pathological pathways, which have subsequent pro-inflammatory responses. It is important to note that all four of these pathological sequels may occur concurrently during the onset and propagation of AMD/AD (10), and recent research has indicated that AD-like pathology may be accelerated by cigarette smoking (11).

Cigarette smoking (CS) has long been known to be an environmental age accelerator (12). It is estimated that by the year 2020 the number of annual tobacco-related deaths will reach 7.5 million, which will account for approximately 10% of all fatalities (13). According to the 2011 WHO statistics (13), tobacco use was the second most significant risk factor for developing a number of non-transmissible diseases. Interestingly, contrasting reports have been published concerning the associations between CS and the risk of developing AD-like pathology or even AD in different populations. One study indicated that while CS renders protection from AD in Western populations, it appears to increase the risk in Asian populations. After adjusting for heterogeneity, heavy smoking (greater than 55.5 packs per year) does appear to consistently increase the risk of developing AD (14). Various animal studies also suggest that CS induces neuropathological changes that can accelerate the progression of AD (15, 16). Furthermore, in the brain matter of cigarette smoke-exposed Lewis rats, the expression of genes encoding for pro-oxidant iNOS, NOX4, dual oxidase1, and p22phox was increased (17). In the hippocampus of cigarette smoke-exposed SD rats, the oxidative DNA damage marker 8-hydroxyguanosine was also increased (15). Oxidative stress can also activate JNK signaling leading to tau phosphorylation and apoptosis. In fact, after 8 weeks of exposure to cigarette smoke, the level of phospho-JNK was increased along with the levels of tau hyperphosphorylation at residues Thr231, Thr205, and Ser404. Reduced expression of various synaptic proteins (e.g., synapsin-1, synaptophysin) and cytoskeleton-related protein (e.g., tubulin, drebrin) were also detected in these cigarette smoke-exposed rats, indicating possible synaptic degeneration and axonal deficits (15). Furthermore, in a mouse model of AD, cigarette smoke was also observed to exacerbate amyloid pathology, resulting in increased amyloid deposition (which subsequently led to the formation of new plaques) and accelerated maturation of the amyloid deposits after only 4 months of exposure (16).

Compared to the conflicting epidemiological evidence linking AD and CS, CS has been shown to be one of the major modifiable risk factors for AMD, almost doubling the risk of developing AMD while also promoting the progression of the disease from the atrophic to the neovascular form (11, 18, 19). These accelerated changes are likely propagated by the toxic compounds found in cigarette smoke, many of which have been shown to induce oxidative stress and/or decrease free radical scavengers (20). In fact, cigarette smoke extract (CSE) has been shown to significantly reduce the viability of RPE-19 cells and primary RPE cells *via* alterations to mitochondrial integrity and increased lipid peroxidation (21). Moreover, CS promotes molecular and pathological changes that may establish an ideal microenvironment for the development of AMD, vascular inflammation, endothelial dysregulation, oxidative damage, toxic damage, as well as histopathological changes in the RPE, Bruch's membrane, and choriocapillaris (11). This hypothesis is supported by previous studies showing that 6 months of exposure to cigarette smoke induces changes in these ocular tissues in a mouse model, which mimic the changes that occur in human AMD (22, 23). Importantly, the smoking-related changes leading to accelerated AMD can, to some extent, be reversed when the patient stops smoking if detected early (19).

Clinically, AMD progression is typically monitored by analyzing the deposition of drusen and changes in the pigmentation of the RPE (24). Aβ assemblies are the most prevalent in retinas with moderate-to-high drusen loads in the advanced stages of AMD (25), which potentially already involve irreversible changes in the patient's vision. While most AMD studies focus on drusen formation in the outer retina, some consideration has been given to early changes occurring in the inner retina. Anti-astrocyte and anti-retinal autoantibodies have been detected in the sera from patients with early forms of AMD, which suggest that changes in the neural retina may also be an early feature of the disease. Abnormalities in the electrooculograms (EOGs) and electroretinograms (ERGs) measured for these retinas also indicate global retinal dysfunction (26). Notably, both normal aging and AMD appear to affect the rod-mediated mfERG measurements (27) and alter neuronal transmission at the postreceptoral level. These functional ERG changes may indicate anatomic and functional plasticity in the synaptic circuity, possibly at the level of photoreceptor-bipolar synapses. In light-damaged rat eyes, which mimic dry AMD, there also appears to be extensive dendritic remodeling and extension of the neurons (28). Moreover, neurons in AMD retinas have the capacity to remodel by sprouting processes and re-forming synaptic complexes with their appropriate targets (29). This remodeling was evident before any evidence of neuronal loss and was accompanied by the reconnection of the presynaptic elements to the postsynaptic bipolar neurons. Thus, if diagnosed early, say before the drusen deposition in the outer retina, AMD could in fact be prevented and/or reversed.

It is in our opinion that in order to diagnose AMD in cigarette smokers early enough to prevent lasting damage, clinicians should focus on the early changes occurring in the retina. To do so, the links between AMD and AD can be exploited to some extent, particularly for the development of diagnostic tools. For

example, the techniques presently being developed and optimized to detect structural and functional changes in AD patients could be invaluable for early AMD diagnosis. In fact, the retinas of AD patient have also been shown to be affected by the disease, with Aβ deposition being detected along with retinal ganglion cell degeneration and decreased thickness of the retinal nerve fiber layer (30), suggesting that the inner retina is primary location of damage in AD patients. Although the location of Aβ42 deposition and the cell type affected in the retinas of AMD patients appear to be different compared to AD patients, similar detection tools could be utilized to diagnose AMD before permanent vision loss. It is essential to conduct longitudinal animal studies investigating the neuronal changes of the retina as well as the RPE, Bruch's membrane, and choidocapillaries together with retinal function tests in order to demonstrate the role these early neuronal changes might also play in AMD pathogenesis. Current animal models can allow us to investigate correlation of these functional changes with the histopathology hallmarks in eyes exposed to CS. Then, we can apply our research findings for non-invasive imaging method to monitor disease progression, contributing to clinical investigations. The information gleaned from these studies may also elucidate additional links between AD and AMD in addition to potentially highlighting biomarkers involved in the early changes occurring during both diseases, in both the brain and retina. Ultimately, we believe it is likely that the link between the brain and retina will play an essential role in aiding researchers in the development of diagnostic tools for CS-induced AMD. The current literature and continued research concerning the early neuronal changes in both the brain and retinas of AD patients and animal models indicate that they could be used as a good model of the early retinal changes in CS-induced AMD. It is our hope that remarking on this phenomenon encourages additional research into the utilization of these previous reports to mediate early diagnosis of AMD (i.e., Aβ, tau, inflammation, microglial activation), and that emerging treatments for AD (anti-amyloid, anti-tau) in conjunction with reduced CS might be useful for the treatment of AMD.

## AUTHOR CONTRIBUTIONS

SY, RC, and KC wrote the manuscript. RC and KC contribute to the original thought. XT and Y-SH give opinion and discuss the content in the revision processes.

## ACKNOWLEDGMENTS

Thanks to Dr. Abby Manthey for critical editorial work on this manuscript. The Studies of cigarette smoking are supported by Seed Funding for incubating Group-based collaborative Research Projects, HKU Alzheimer's Disease Research Network under Strategic Research Theme of Ageing and by a generous donation from Ms. Kit-Wan Chow.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 08 August 2016
doi: 10.3389/fneur.2016.00127

# Amyloidosis in Retinal Neurodegenerative Diseases

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As a part of the central nervous system, the retina may reflect both physiological processes and abnormalities related to pathologies that affect the brain. Amyloidosis due to the accumulation of amyloid-beta (Aβ) was initially regarded as a specific and exclusive characteristic of neurodegenerative alterations seen in the brain of Alzheimer's disease (AD) patients. More recently, it was discovered that amyloidosis-related alterations, similar to those seen in the brain of Alzheimer's patients, also occur in the retina. Remarkably, these alterations were identified not only in primary retinal pathologies, such as age-related macular degeneration (AMD) and glaucoma, but also in the retinas of Alzheimer's patients. In this review, we first briefly discuss the biogenesis of Aβ, a peptide involved in amyloidosis. We then discuss some pathological aspects (synaptic dysfunction, mitochondrial failure, glial activation, and vascular abnormalities) related to the neurotoxic effects of Aβ. We finally highlight common features shared by AD, AMD, and glaucoma in the context of Aβ amyloidosis and further discuss why the retina, due to the transparency of the eye, can be considered as a "window" to the brain.

**Keywords: Alzheimer's disease, age-related macular degeneration, glaucoma, neurodegeneration, synaptic and mitochondrial dysfunction, micoangiopathy, neuroinflammation**

OPEN ACCESS

***Edited by:***
*Raymond Chuen-Chung Chang, University of Hong Kong, Hong Kong*

***Reviewed by:***
*Varun Kesherwani, University of Nebraska Medical Center, USA*
*Abby Manthey, University of Hong Kong, Hong Kong*

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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *03 May 2016*
***Accepted:*** *27 July 2016*
***Published:*** *08 August 2016*

***Citation:***
*Masuzzo A, Dinet V, Cavanagh C, Mascarelli F and Krantic S (2016) Amyloidosis in Retinal Neurodegenerative Diseases. Front. Neurol. 7:127. doi: 10.3389/fneur.2016.00127*

## INTRODUCTION

Pathological alterations, such as synaptic dysfunctions, neuronal cell loss, inflammatory responses, microvasculature abnormalities, mitochondrial failure, and oxidative stress, have been associated with amyloid-beta (Aβ) in the brain. However, similar pathological alterations have more recently also been reported in the retina where they may mirror analogous events occurring in the brain (1). The present review will focus on these aforementioned aspects of Aβ's deleterious effects but does not have the ambition to cover all aspects of Aβ cytotoxicity. For instance, the issues related to aberrant Aβ clearance will not be discussed here since they have been recently extensively reviewed elsewhere [e.g., Ref. (2)].

Retinal accumulation of Aβ is broadly recognized as being involved in amyloidosis-associated neurodegeneration. Pathological hallmarks of amyloidosis are related to the accumulation of specific types of proteins, including Aβ, prone to oligomerize with a high content of beta (β)-sheet structures (3). Among the neurodegenerative diseases related to Aβ amyloidosis, Alzheimer's disease (AD) is certainly the best known and the most studied. More recently, it has been recognized that Aβ-related amyloidosis also occurs during glaucoma and age-related macular degeneration (AMD). Historically and up to very recently, AD was considered as an exclusively cerebral disorder, while glaucoma and AMD were regarded as neurodegenerative disorders specific to the retina. However,

it is increasingly clear that AD-like pathological alterations seen in the brain also occur in the retina (4), where they may even start earlier. Conversely, the pathological phenomena observed in glaucoma, for example, are associated with neurodegeneration of selected brain areas (5). Altogether, this new evidence suggests that the retina may be used as the "window" to the brain for the study of the earliest pathophysiological changes involved in neurodegeneration. This attractive idea is behind different aspects of amyloidosis that will be discussed here.

Parkinson's disease (PD), which shares many features of Aβ-amyloidosis with AD, glaucoma, and AMD, will not be discussed here, and we recommend a number of excellent and exhaustive reviews on this topic (6, 7). Indeed, although PD is considered an amyloidosis-associated disease, involving the accumulation of both Aβ and α-synuclein, the relevant fibrils have not been identified in the PD retina (8). This is in sharp contrast with the presence of Aβ plaques, identical to those found in AD-vulnerable brain areas that have been identified in the retina (9, 10). Furthermore, Aβ-amyloidosis seen in PD is sometimes considered as an epiphenomenon to the oligomerization of α-synuclein into structures known as Lewy bodies. Consequently, rigorous analysis of alterations specific to Aβ-amyloidosis in PD would require a systematic comparative follow-up of cohorts composed of "mixed" PD (displaying both α-synuclein and Aβ-amyloidosis) and "pure" PD (displaying exclusively α-synuclein amyloidosis). Such studies, similar to the one reported by Bertrand and colleagues (11), are still relatively scarce. Finally, there is no consensus about the precise type of pathological alterations in the PD retina, since thickening (12), thinning (13), and absence of change (14) in the retinal nerve fiber layer (RNFL) have all been reported. The analysis of retinal Aβ-amyloidosis in PD would therefore be more complicated. By consequence, this review will focus only on AD, glaucoma, and AMD.

## BIOLOGY OF AMYLOID-β AND ITS PRECURSOR APP

Amyloid precursor protein (APP), a type 1 transmembrane glycoprotein, belongs to a family of proteins, which in mammals include APP-like protein-1 (APLP1) and APP-like protein-2 (APLP2) (15). Despite the widespread expression of the APP gene in mammalian and non-mammalian cells, the physiological role of APP is still unclear. APP-related mRNA has been found not only in the nervous system but also in the immune system, muscles, and other organs, such as the pancreas, lung, and kidney (16, 17). Alternative splicing of APP mRNA gives rise to multiple isoforms, which are differentially expressed among various tissues and different stages of development. In particular, APP is upregulated during brain development, and specific APP variants are associated with neurite outgrowth and synaptogenesis (18, 19).

There are three major APP isoforms, APP770, APP751, and APP695, which are all generated from the alternative splicing of exons 7 and/or 8. APP695 is mainly neuronal, whereas the other two variants are principally non-neuronal (20). APP polypeptides undergo posttranslational modifications (such as glycosylation and phosphorylation) and are subsequently addressed to the plasma membrane *via* the constitutive secretory pathway (**Figure 1A**). Successively, APP is internalized through clathrin-mediated endocytosis and reaches the endosomal system. Part of endosomal APP is recycled to the cell surface, whereas another conspicuous part is degraded in lysosomes (21, 22). In the steady state, APP is preferentially localized in the Golgi and in the trans-Golgi network, and only a tiny fraction is localized on the cell surface.

Amyloid precursor protein can be posttranslationally processed through two distinct pathways [reviewed in Ref. (23)] (**Figure 1B**). The amyloidogenic pathway involves sequential cleavage steps by β-secretase and γ-secretase, which generates Aβ. The second pathway, which is predominant, involves sequential cleavage steps by α-secretase and γ-secretase but does not yield Aβ. Indeed, α-secretase cleavage occurs within the Aβ peptide region, preventing Aβ formation. It has been shown that proteases belonging to the A-disintegrin and metalloproteinase (ADAM) family have α-secretase activity (24–26). Since ADAMs are cell surface proteins, α-secretase cleavage likely occurs at the level of the plasma membrane and involves the membrane pool of APP (27). α-secretase cleavage leads to the formation of an amino-terminal fragment called secreted APP (sAPP)α and a carboxy-terminal fragment called CTF83. β-secretase is a type 1 transmembrane protease, and its cleavage leads to the formation of sAPPβ and CTF99. In converse to α-secretase cleavage, β-secretase cleavage occurs mainly in endocytic vesicles and not at the cell surface, where both β-site APP cleaving enzyme-1 (BACE1) and APP are swiftly recycled. The first cleavage step is followed by γ-secretase cleavage in both pathways. The latter is a protein complex composed of at least four proteins: presenilin (PS) 1 or 2, nicastrin, presenilin enhancer 2 (Pen 2), and anterior pharynx defective 1 (Aph-1) (28). γ-secretase processes CTF83 and CTF99, yielding the APP intracellular domain (AICD) plus the fragment p3 and AICD plus Aβ, respectively. Aβ peptides of different lengths, from 38 to 43 amino acids, can be generated by γ-secretase cleavage; however, Aβ1–42 and Aβ1–40 are considered to be the most relevant forms to amyloidosis.

Since APP undergoes sequential cleavage steps, it has been difficult to distinguish the physiological role of APP from those of its cleavage products. Generally, the role of APP in the brain is regarded as beneficial and often associated with its cleavage product, sAPPα. It has been shown that APP promotes cell proliferation, neuronal stem cell differentiation, neurite outgrowth, synaptogenesis, cell adhesion, and regulates long-term potentiation (LTP). APP-KO mice are viable and fertile, suggesting that APP – or its products – are not essential for development or alternatively, are part of a network of proteins with redundant functions (29). However, APP-deficient mice present various abnormalities, such as reduced body and brain size, hypersensitivity to seizures, and impaired learning and LTP. These phenotypes are rescued by the introduction of sAPPα in APP-deficient mice, suggesting that sAPPα may play an important role in brain development and function (30).

Compared with sAPPα, little is known about the putative physiological roles of other cleavage products from the non-amyloidogenic and amyloidogenic pathways. However, it has been proposed that Aβ may regulate synaptic activity, although controversial results have been reported on its beneficial versus

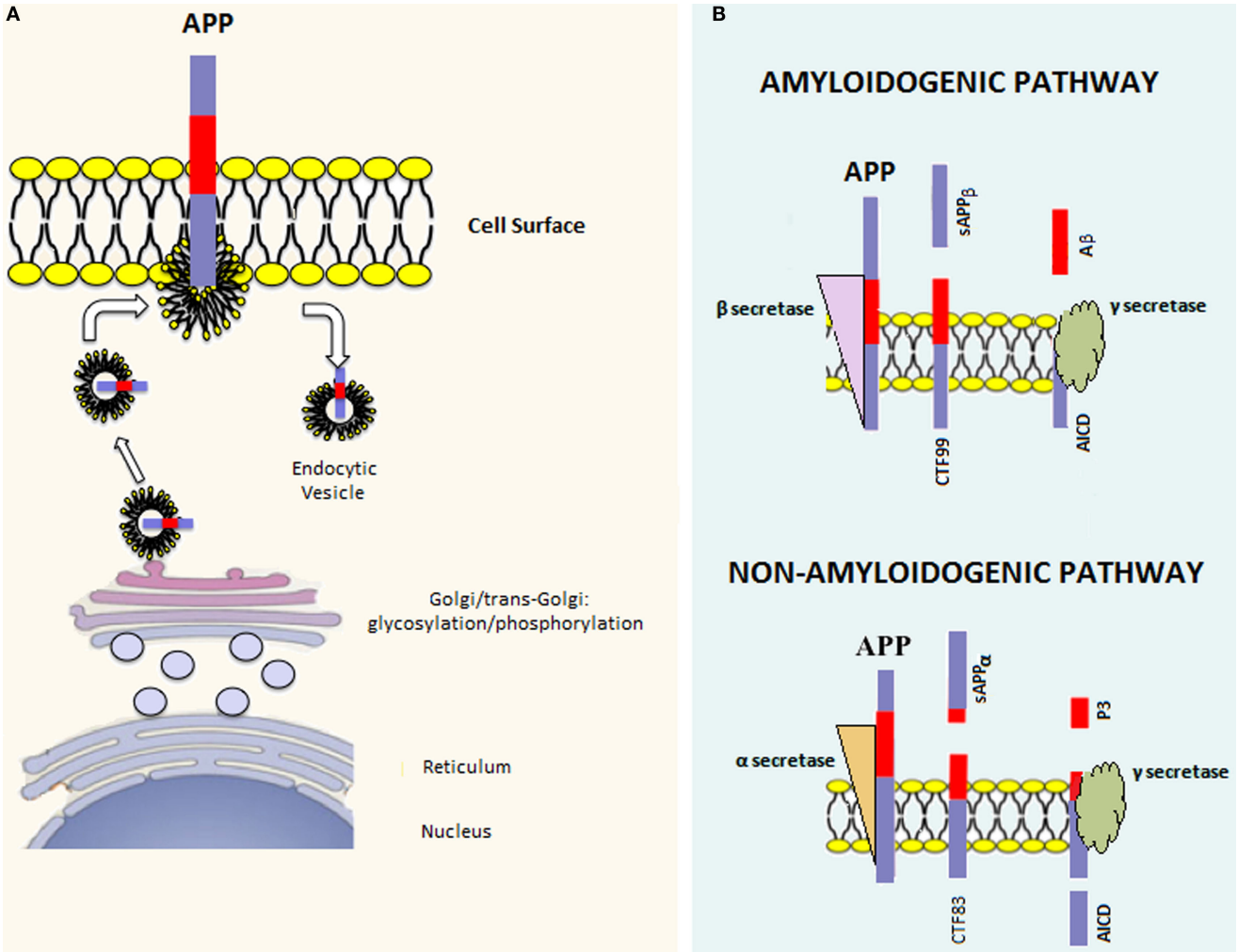


**FIGURE 1** | **APP processing**. **(A)** Once the APP mRNA is translated, the nascent polypeptide undergoes posttranslation modifications (e.g., glycosylation and phosphorylation) in the endoplasmic reticulum and Golgi apparatus. Afterward, the mature APP protein is addressed to the cell surface through the constitutive secretion pathway. At this point, APP is internalized in endocytic vesicles. Endosomal APP can be recycled to the cell surface or degraded through the lysosomal system. **(B)** APP can be processed through two distinct pathways. The amyloidogenic pathway involves cleavage by β-secretase, which leads to the formation of the carboxy-terminal fragment 99 (CTF99) and soluble APPβ (sAPPβ). This cleavage is followed by a second cleavage mediated by γ-secretase that leads to the formation of the APP intracellular domain (AICD) and Amyloid-β (Aβ). On the other hand, the non-amyloidogenic pathway involves the sequential cleavage first by α-secretase, which leads to the formation of the carboxy-terminal fragment 83 (CTF83) and soluble APPα (sAPPα), followed by γ-secretase cleavage, which leads to the formation of AICD and P3.

deleterious effects (31, 32). In addition, Aβ may be involved in the control of cholesterol transport (33) and lipid homeostasis (34). For instance, direct activation of sphingomyelinase and inhibition of hydroxymethylglutaryl-CoA reductase (HMGR) by Aβ1–42 and Aβ1–40 have been demonstrated (35). The question of the physiological role of Aβ remains open, and further studies are clearly needed in this field.

## AMYLOID-β AND ITS PRECURSOR IN THE EYE AND RETINA

The retina is a highly specialized neurosensory tissue, which lines the back of the eye. It is an integral part of the brain comprising six different types of neuronal cells and two types of macroglia cells: retinal Müller glial cells and astrocytes. Retinal and central nervous system (CNS) neurons are derived from common progenitors (36). Differentiated retinal neurons are organized into a well-defined laminar structure and are distributed into three cell and two synaptic layers. The outer segment of the retina is populated by two different types of photoreceptors: cones and rods, which are able to detect light and form the outer nuclear layer (ONL). The detected light signal is transmitted to the cells located at the inner nuclear layer (INL), mainly the bipolar cells followed by retinal ganglion cells (RGCs), either directly or indirectly *via* type II amacrine cells. The latter, together with horizontal cells, modulate glutamatergic neurotransmission along the main synaptic axis comprising photoreceptors, bipolar, and ganglion cells. The principal function of the INL cells is to integrate and regulate

the signal input. The RGC axons converge into the optic nerve fibers, which convey the signal to the visual cortex (37).

To date, the physiological roles of APP in the retina have not been extensively investigated, although a consensus has been met about its expression by retinal pigmented epithelial (RPE) cells in the healthy retina (38). The role of APP in the development of the mouse retina has been recognized such that APP is required for the full differentiation of the AII subtype of amacrine cells. Similar to its role in the brain, APP may be implicated in retinal synaptogenesis. Indeed, APP participates in the developmental determination of the inner plexiform layer (IPL), where amacrine cells synapse to bipolar and ganglion cells (39). Concerning the physiological role of APP in adult mice, it has been shown that APP regulates inner retinal layer function. Indeed, APP-KO mice display alterations in the rod and cone pathways. However, these mice do not present any major deficits in visual function; therefore, APP is not likely a required factor (40). Among all retinal neurons, at least in the rabbit, ganglion cells are the sole cells able to synthesize and express APP on their plasma membrane in the absence of any pathological insult (41). In the human retina, APP expression is age-dependent and was revealed in RGC neurons and the RNFL (42).

Concerning Aβ, there is no published data on its putative physiological role in the retina. Of interest, the expression of BACE1 has been recently reported in the blood–brain barrier endothelial cells of mouse, bovine, and human origin, thus suggesting putative local production of Aβ in cerebral blood vessels (43). It remains unknown whether retinal vessel endothelial cells display analogous BACE1 expression. By contrast, BACE1 expression has been reported in the plexiform layer of the rat retina pointing to its synaptic localization (44).

The other parts of the eye have been much less studied in terms of the expression and function of APP and its cleavage products. However, both APP and the proteolytic enzymes involved in its cleavage were found to be expressed in some other eye compartments. For instance, APP and the secretases involved in its processing were identified in the lens (45). Similarly, Aβ was identified both in the lens (46) and in the vitreous fluid (47).

## PATHOLOGICAL ACCUMULATION OF AMYLOID-β: AMYLOIDOSIS, AMYLOIDOPATHY, AND AMYLOIDOGENESIS

Different terms have been associated with the pathological accumulation of Aβ, with amyloidosis historically being used first. Amyloidosis is a broad term designating a metabolic disease characterized by the extracellular accumulation of globular or natively unfolded or misfolded amyloidogenic polypeptides. Amyloidogenic polypeptides contain a high proportion of β-sheets and have a great propensity to aggregate into highly organized and kinetically stable amyloid fibrils, amorphous aggregates, or oligomers. To date, more than 20 precursor proteins of fibrils (including APP) have been identified in systemic and localized amyloidosis (3). A remarkable property of these fibrils is that, independent of the type of the precursor protein, they are all 80–100Å in width. Furthermore, these fibrils organize in a tridimensional β-pleated sheet conformation with the direction of the polypeptide backbone perpendicular to the fibril axis (cross-beta structure). Another remarkable characteristic of amyloidogenic peptides and derived aggregates is their affinity for the Congo red stain (48). The Aβ-related amyloidopathies consist of increased intra- and/or extracellular accumulation of Aβ and deposition of Aβ in the form of insoluble material, such as amyloid plaques or drusens. Several disorders are associated with amyloidopathies, and most of them are neurodegenerative diseases (e.g., AD, PD, polyglutamine diseases, prion disorders, and AMD).

Amyloid-beta is produced *via* the amyloidogenic pathway of APP processing. However, the mechanisms by which this pathway may take over the non-amyloidogenic pathway are poorly understood, especially considering that both pathways coexist in physiological conditions (49). Many genetic and epigenetic factors may be involved, but the evidence points to an increase in the ratio of β- over α-secretase activity as a trigger. This change in the subtle balance between secretase activities in physiological conditions might be associated with the positive control of β-secretase activity by its substrate APP and directly related to APP overexpression and subsequent increase in Aβ production (50). Over the course of normal aging, Aβ is deposited subretinally in the mouse and human retina (51). With age, Aβ accumulates at the interface of the RPE and the photoreceptor outer segment tips. This finding is consistent with increased Aβ1–42 secretion by aged human RPE cells (52). As Aβ accumulates subretinally, microglial cells in normal aged mice become bloated with cellular debris and Aβ (51). The accumulation of Aβ in the subretinal space might contribute to the 23–30% reduction in photoreceptors that occurs over human lifetimes (53).

## AMYLOID-β AGGREGATION AND TOXICITY

An increase in Aβ production above normal physiological levels yields cytotoxicity. Among most common Aβ species (i.e., 1–40 and 1–42 amino acid-containing isoforms), Aβ1–42 is considered the most neurotoxic as it is more prone to oligomerization (54). The amyloid aggregation pathway is still poorly understood and several intermediates are likely involved. Small soluble Aβ monomers can interact to form Aβ oligomers in the extracellular space. Aβ oligomers aggregate to form larger fibrils, which in turn aggregate to form extracellular plaques. The mechanisms of Aβ toxicity are still unclear, and different hypotheses have been proposed. According to the original "amyloid-β cascade hypothesis," insoluble amyloid fibrils are the main molecular culprit underlying toxicity (55). More recently, this hypothesis has been revised to the "oligomeric amyloid-β hypothesis" (56). It is currently believed that the most toxic intermediates are small oligomers (with degree of polymerization lower than 10), also known as amyloid-β diffusible ligands (ADDL) or protofibrils. The latter have a bigger diffusivity and a larger surface-to-volume ratio that leads to the exposure of hydrophobic patches (57). However, it is not yet clear which oligomeric species is "the most" toxic

since dimers/trimers (58), tetramers (59), and duodecamers such as Aβ*56 (60) have all been considered as plausible candidates depending on the paradigm (*in vivo*, *in vitro*) or species (murine, human) studied.

Soluble Aβ oligomers, although they are certainly not involved in all the aspects of AD, are still regarded as key initial triggers of pathogenesis (61). The bioactive pool of soluble Aβ comprises two fractions: the first is generated in the endosomal compartment and secreted into the extracellular space by exocytosis and the second is intracellular and has been found in both AD patients and animal models of the disease (62, 63). Cellular mechanisms by which soluble oligomers exert neurotoxic effects are multifaceted, involving synaptotoxicity and mitochondrial dysfunction likely related to oxidative stress and metabolic impairment. Insoluble Aβ aggregates also contribute to Aβ toxicity either directly through the release of soluble oligomers (64) or indirectly *via* adaptive cellular responses, such as glial and endothelial activation, which can yield neuroinflammation (65) and Aβ-related angiopathy (66), respectively.

## Amyloid-β and Synaptic Dysfunction

One of the prominent facets of Aβ toxicity concerns synaptic loss (67). This toxicity may be related to a deviation from the Aβ-associated modulation of synaptic excitability under physiological conditions (31). Indeed, increased synaptic activity may enhance Aβ release at the synaptic level, reducing excitatory postsynaptic transmission. In particular, it has been shown both *in vitro* and *in vivo*, that Aβ oligomers reduce glutamatergic synaptic transmission by decreasing the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl D-aspartate (NMDA) receptors at the synapse (68–71) (**Figure 2A**). A decrease in AMPA receptors by Aβ has been related to increased phosphorylation of the $Ca^{2+}$-permeable subunit, GluR2, and a subsequent increase of intracellular $Ca^{2+}$ levels (72). A decrease in NMDA receptors by Aβ involves a similar mechanism *via* dephosphorylation of the NR2B subunit and subsequent increase in receptor endocytosis (73). Thus, Aβ is part of a refined regulatory circuit in which intermediate levels of Aβ are correlated with a physiological increase in presynaptic activity, whereas lower or higher Aβ levels are correlated with reduced presynaptic and postsynaptic transmission, respectively (74). Likely, Aβ differentially affects synaptic activity, depending on synapse type, neuron type, and/or brain region, leading to the imbalance and instability of neuronal networks (75). At the cellular level, Aβ-mediated alterations involve a shift toward increased excitability manifesting in a decreased resting potential of the neuronal membrane (76). Similarly, the addition of exogenous Aβ oligomers to hippocampal neurons induced hyperpolarization of the action potential (AP) threshold and decreased after-hyperpolarization (AHP), both compatible with an increase in neuronal excitability (77).

Of note, the vast majority of the above-discussed data has been obtained *in vitro*, by treating cerebral (hippocampal or cortical primary) neurons with soluble Aβ oligomers. Analogous data for retinal neurons are scarce, although it has been reported that intravitreal injection of Aβ triggers acute photoreceptor cell death and delayed RGC apoptosis (78). The latter likely involves an indirect mechanism *via* the activation of Müller cells (78). Finally, a similar Aβ challenge by intravitreal injection resulted in an impaired pattern of acetylcholine, γ-aminobutyric acid (GABA), and serotonin neurotransmitter expression with catecholaminergic markers being relatively unaffected (79).

## Amyloid-β and Mitochondrial Dysfunction

Mitochondrial dysfunction is a common feature of various neurodegenerative diseases and causes alterations in mitochondrial respiratory enzyme complex activities, oxidative stress, opening of mitochondrial permeability transition pores (mPTPs), and enhanced apoptosis (80). In the brain, intracellular Aβ has been associated with axonopathy and apoptosis initiation (81, 82). Moreover, in neurons, mitochondrial dysfunction is also associated with increased susceptibility to excitotoxicity (i.e., cell death caused by excessive stimulation of neurons by excitatory amino acids, such as glutamate) (83).

Soluble Aβ peptides have been found in different organelles, and their deleterious effects are largely due to their accumulation within mitochondria. Indeed, intracellular Aβ inhibits the activity of different mitochondrial respiratory enzymes, causes decreased ATP production, and increases the production of reactive oxygen species (ROS) (84–87) (**Figure 2B**). Moreover, Aβ induces mitochondrial dysfunction by interacting with the Aβ-binding protein known as Aβ-binding alcohol dehydrogenase (ABAD), which is present on the mitochondrial membrane (88). In addition, Aβ accumulation impairs the permeability of mitochondrial membranes leading to the opening of mitocholdrial $Ca^{2+}$ channels and mPTPs as well as the enhancement of cytochrome *c* (Cyt*c*) release (89). At the structural level, accumulation of soluble Aβ impairs mitochondrial fusion and fission and triggers abnormalities in mitochondrial trafficking, morphology, and degradation [reviewed in Ref. (90)].

In the retina, intraocular injection of respiratory complex (I, III, and IV) inhibitors or Aβ fibrils yields induction of BACE1 expression and activity, suggesting that Aβ-mediated mitochondrial respiratory inhibition and oxidative stress facilitate BACE1 expression (44). Interestingly, subretinal injection of Aβ oligomers resulted in RPE cell hypertrophy without triggering apoptosis but yielded a significant amount of delayed photoreceptor death (91).

## Amyloid-β and Glial Activation

The presence of misfolded proteins and their aggregates causes an alteration in the receptor–ligand interactions that modulate both microglia and astroglia activity. Both microglia and astroglia release cytokines, nitric oxide, and other cytotoxic molecules after Aβ exposure (**Figure 2C**). Astroglia regulate synapse formation and function in addition to participating in the tripartite synapse (92). It was shown that Aβ upregulates NFκB in astrocytes, leading to C3 release (93). The latter binds the neuronal G-protein-coupled receptor C3aR, inducing dendritic structural alterations and synaptic dysfunction. C3 also interacts with microglial C3aR causing alterations in cognitive function and impairment of Aβ phagocytosis (94). Moreover, the exposure of astroglia to Aβ, favors astrogliosis, a process that leads to molecular and functional changes in astrocytes and is

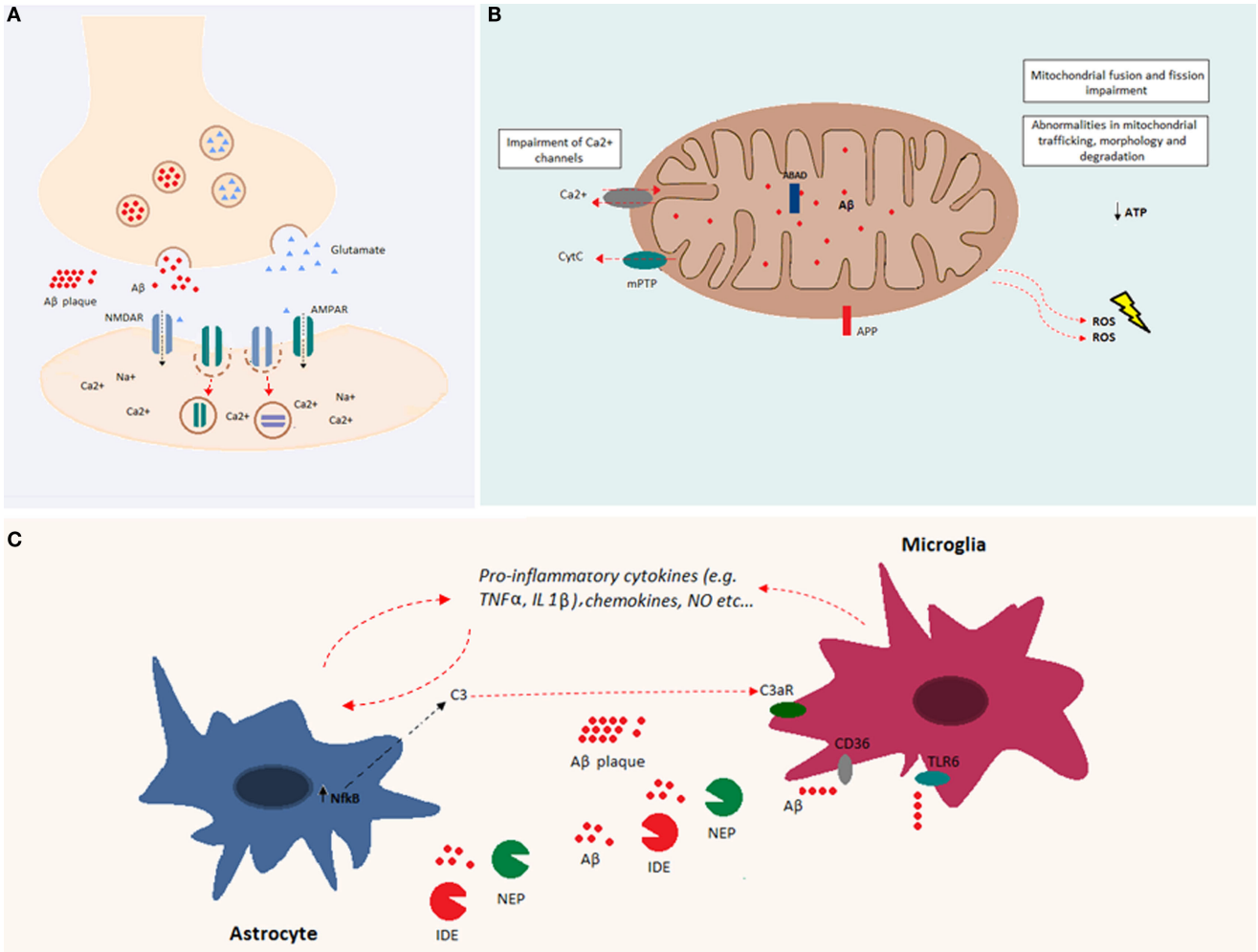


**FIGURE 2** | **Overview of Aβ cellular effects**. **(A)** Aβ is implicated in synapse loss. Increased Aβ at the synapse reduces excitatory postsynaptic transmission. Indeed, Aβ oligomers reduce glutamatergic synaptic transmission by decreasing the number of both AMPA and NMDA receptors at the postsynaptic membrane. **(B)** Aβ accumulation within the mitochondria causes impairments in fusion and fission and abnormalities in mitochondrial trafficking, morphology, and degradation. Both APP and Aβ can interact with mitochondrial membranes. Aβ, by interacting with mitochondrial respiratory enzymes, causes decreased ATP production and increased reactive oxygen species (ROS) production. In addition, Aβ binds the Aβ-binding alcohol dehydrogenase (ABAD), increasing its deleterious effects in mitochondrial function. Mitochondrial $Ca^{2+}$ channels are impaired by Aβ, and mitochondrial permeability transition pore (mPTP) opening gives rise to the enhancement of cytochrome *c* (Cytc) release. **(C)** Aβ accumulation induces glial activation. Astrocytes and microglia release cytokines, chemokines, and nitric oxide (NO) after exposure to Aβ. Increased levels of NFκB in astrocytes induce the release of C3, which binds the C3a receptor, impairing microglia-mediated Aβ phagocytosis. Both microglia and astrocytes release Aβ-degrading proteases, such as neprilysin and insulin-degrading enzyme. Aβ fibrils are degraded through microglia-dependent phagocytosis, triggered by the ligation of Aβ to microglia receptors (e.g., CD36 and TLR-6).

implicated in different brain diseases (95). Furthermore, astroglia play an important role in Aβ clearance. Indeed, astrocytes are able to bind to and degrade Aβ and release extracellular Aβ-degrading proteases (e.g., neprilysin, insulin-degrading enzyme, angiotensin-converting enzyme-1, and endothelin-converting enzyme-2) (96, 97). On the other hand, microglia are phagocytic cells ubiquitously distributed in the brain. Microglia play important roles in the maintenance and plasticity of neuronal circuits, in the surveillance for pathogens or cell debris, and in tissue maintenance (98–100). Aβ oligomers and fibrils are able to bind microglia surface receptors, such as cluster of differentiation-36 (CD36), toll-like receptor (TLR)-4, and TLR-6, leading to their activation (101, 102). Activated microglia release proinflammatory cytokines and chemokines (102, 103). Consequently, extracellular proteases (in particular, neprylysin and the insulin-degrading enzyme) are released and give rise to enzymatic degradation of soluble Aβ (104). In addition, receptor ligation triggers the activation of microglial-dependent phagocytosis of Aβ fibrils and their degradation through the endolysosomal pathway. Aβ accumulation itself leads to increased release of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNFα), interleukin (IL)-1α, and IL-1β (105, 106). The massive release of proinflammatory cytokines might be associated with impairment of synaptic transmission by suppressing LTP

(100). It has also been shown that there is a positive feedback loop between TNFα and Aβ, since TNFα is able to induce Aβ production by increasing BACE1 expression and γ-secretase activity (107, 108). In addition, the use of TNFα inhibitors leads to a decrease in APP processing and Aβ (109). Similarly, IL-1β increases Aβ production by increasing γ-secretase activity (107). Even though the early activation of astroglia and microglia is beneficial and leads to Aβ clearance, in a pathological context, the sustained activation of these cells may induce a positive feedback loop between APP processing and inflammation, which is deleterious (100). Indeed, inflammation is a consequence of Aβ accumulation, and as a result, inflammation contributes directly to the pathogenesis and progression of the disease.

## Amyloid-β and Blood Vessels

The pathophysiological cause and consequence of the accumulation of Aβ and/or its precursor APP in the brain and the retina remain poorly understood. Twenty years ago, it was reported that coincident APP and B-cell lymphoma-2 (Bcl-2) induction may play a role in rat retinal cell survival after optic nerve and vascular injury. The underlying mechanism involves APP induction selectively in either activated astrocytes (Müller cells) or neurons (110). Microinjection of Aβ into the adult zebrafish eye triggers an increase in endothelial tip cells and a subsequent increase in the capillary bed density without affecting larger arterial vessels (111). In this light, the recent discovery of BACE1 expression in endothelial cells (indicating local cleavage of APP to Aβ in the blood–brain barrier in mice, bovine, and humans) has attracted much interest (43). Indeed, BACE1 appears to be a critical regulator of retinal homeostasis since genetic invalidation of BACE1 in mice yields retinal thinning, apoptosis, reduced retinal vascular density, and increased accumulation of the age pigment, lipofuscin (112). The use of BACE1 inhibitors for therapeutic purposes should therefore be carefully evaluated for the putative impairment of retinal homeostasis.

Some aspects of endothelial BACE1 regulation have been elucidated, such as its induction in the presence of reduced levels of microRNA-195 (miR-195) in hypoperfusion/hypoxic conditions (113). This BACE1 induction is associated with reduced occludin expression in tight junctions of cerebral blood vessels (114). The cellular mechanism behind the deleterious effects of Aβ on cerebral vessel endothelial cells involves activation of the cationic $Ca^{2+}$-permeable channel transient receptor potential melastatin-2 (TRPM-2) and intracellular $Ca^{2+}$ overload (115). In fact, the Aβ-mediated decrease in zonula occludin-1 (ZO-1) expression is attenuated by neutralizing antibodies against receptor for advanced glycation end-products (RAGE) and inhibitors of calcineurin, suggesting that the Aβ–RAGE interactions disrupt tight junction proteins *via* the $Ca^{2+}$-calcineurin pathway (116).

# Aβ AMYLOIDOSIS-RELATED RETINAL NEURODEGENERATIVE DISEASES

Accumulation and aggregation of Aβ is a common denominator of a number of neurodegenerative diseases. Some of them primarily affect the eye/retina (AMD, glaucoma), while others display more specific cerebral manifestations, such as AD and PD. However, evidence is accumulating in support of retinal alterations that may reflect the cerebral neurodegeneration seen in AD and PD patients.

## Alzheimer's Disease

Alzheimer's disease is the main cause of dementia and the most common neurodegenerative disorder in the elderly. It is characterized by cognitive, memory, and language impairments leading to a complete loss of executive functions at the advanced stages (https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf). From a histophatological point of view, two main hallmarks of AD are Aβ plaques and neurofibrillary tangles (NFTs). The latter are mainly composed of hyperphosphorylated tau protein, a microtubule-associated protein (MAP) essential for the maintenance of neuronal polarity and structure (117). It has been shown that Aβ accumulation leads to disassembly of tau from the microtubules and promotes its hyperphosphorylation (118, 119). The hyperphosphorylation of tau and its subsequent oligomerization results in the formation of intracellular NFTs. Ultimately, cytotoxic NFTs act in synergy with oligomeric Aβ and lead to synaptic dysfunction and axonal loss (120, 121).

### AD Pathology in the Brain

Functional alterations associated with AD have been extensively studied in the brain at different levels (network/circuit, cellular, subcellular, and molecular) of organization.

#### *Synaptic Dysfunction*

Amyloid-beta oligomers reduce glutamatergic synaptic transmission by decreasing the number of both AMPA and NMDA postsynaptic receptors (68–71). Besides, a small increase in Aβ has been correlated with increased presynaptic transmission, implicating the activation of α7-nicotinic acetylcholine receptors (nAChR) (32, 122). These synaptic dysfunctions coincide with dysregulation of both LTP and long-term depression (LTD), which are attenuated and enhanced, respectively. Such functional impairments are accompanied with a collapse of dendritic spines and synaptic loss (69, 70, 123). Importantly, AD is characterized by aberrant excitatory network activity and synchronization, which leads to dysfunction of learning and memory circuits and subsequent cognitive decline (124).

#### *Mitochondrial Dysfunction and Oxidative Stress*

Mitochondrial dysfunction is an early event in AD pathogenesis (87). Both APP (125) and Aβ (126) are targeted to mitochondria. Mitochondrial Aβ accumulation has been clearly demonstrated both in AD patients and in transgenic AD mouse models (127, 128). However, the precise mitochondrial actions of Aβ are still poorly understood. In particular, it is unknown whether mitochondrial translocation of intracellular Aβ is required for the inhibitory effects on mitochondrial membrane potential (MMP) and ATP levels recently demonstrated in a transgenic mouse AD model (TgAPP/PS1) (129). Besides, it has been suggested that Aβ cooperates synergistically with tau in the impairment of oxidative phosphorylation (86). Indeed, several mitochondrial

respiratory enzymes were found to be altered in AD, leading to impairments in energy metabolism (130), but the cause–effect relationship between these impairments and Aβ has not been entirely elucidated.

### *Neuroinflammation*

Prominent glial cell activation and related neuroinflammation are seen at the advanced stages of AD and likely play a pivotal role in AD progression (100). The aggregation of both Aβ and tau protein leads to the activation of microglia and astroglia, which are consistently found surrounding Aβ deposits in postmortem AD brains (131–133). More recently, positron emission tomography (PET) brought additional *in vivo* evidence for AD-associated cerebral microgliosis (134).

Accordingly, evidence of neuroinflammation was present in all studied AD mouse models (65). In particular, a prominent induction of TNFα and shift from phagocytic M2 toward the cytotoxic-like M1 microglia phenotype has been reported in the hippocampus at the overt stages of AD pathology in TgAPP/PS1 mice, and this effect was reproduced by treating microglia cultures with oligomeric Aβ (135). This upregulation is accompanied by the coincident induction of another major proinflammatory cytokine, IL-1β, not only in the TgAPP/PS1 mouse (136, 137) but also in Tg2576 (138), 3xTg (139), and TgCRND8 (140) mice. Most importantly, all these studies confirmed consistent and concomitant microglia and astrocyte activation.

The microglia M1-like activation state is characterized by uncontrolled proinflammatory cytokine and chemokine secretion, inefficient Aβ phagocytosis, and TLR activation, which further fuels neuroinflammation (65). Among the relevant cytokines and chemokines, monocyte chemoattractant protein (MCP-1) was repeatedly implicated. The membrane pore-forming capacity of Aβ oligomers has also been related to neuroinflammation (141). Classically, deleterious neuroinflammatory environments exacerbate AD-related pathological alterations and have been consistently involved in AD progression. However, evidence is mounting to suggest that neuroinflammation likely also occurs before significant Aβ accumulation (142). Moreover, proinflammatory alterations related to the upregulation of TNFα in the context of partial microglia activation may occur even before Aβ accumulation (143).

### *Amyloid Microangiopathy*

Microangiopathy, which comprises a host of pathological alterations in the small blood vessels (arterioles, venules, and capillaries), is closely related to cerebral small vessel disease (CSVD). These are heterogeneous pathological conditions that include cerebral blood flow deregulation, endothelial activation, and blood–brain barrier disruption (144).

Such pathological alterations are also found in cerebral amyloid microangiopathy (145). This particular form of microangiopathy results from Aβ deposition within the walls of capillaries or immediately in the adjacent brain parenchyma (145, 146). According to an emerging concept, these lesions may play a causal role in cerebral dysfunction and precede AD-related cognitive impairments (146). Remarkably, although Aβ accumulates selectively in arterioles, the cortical vasculature network appears to be altered in TgCRND8 mice. Extensive structural and functional alterations were observed, including vessel coiling and looping, increased tortuosity of the venules (but not arterioles), and altered microvascular network cerebral blood flow response to hypercapnia (147).

Another prominent feature of AD-related amyloid microangiopathy is the presence of microbleeds. In the Tg2576 mouse model of AD, these microbleeds are due to leakage or rupture of microvasculature in brain regions affected by vascular amyloid deposits (148). Such microbleeds may be related to the upregulation of BACE1 observed in endothelial cells of the blood–brain barrier in another mouse AD model (43) as well as AD patients (114). The knockdown of miR-195, which regulates BACE1 expression at least in endothelial cells, yields increased tau phosphorylation at Ser202/Thr205, Ser262, Thr231, and Ser422, as well as Cdk5/p25 activation in the rat hippocampus (113).

## AD Pathology in the Retina

The accumulation of Aβ and its deposition into Aβ plaques have been found in postmortem retinas from AD patients (9). In addition, visual disturbances are common in AD, and they may be due to local retinal abnormalities rather than exclusively related to central, visual cortex alterations (149). However, the molecular mechanisms underlying these visual disturbances and the role that Aβ may play in the retina are still largely unknown. Structural abnormalities identified in retinas of AD patients include reduced number of optic nerve fibers and altered thickness of the parapapillary and macular RNFL (150, 151). These structural changes likely reflect retinal neurodegeneration, such as RGC death (152), and are further associated with optic nerve damage (153).

Consistently, Aβ plaques have also been found in the retina of AD transgenic mouse models (9). Retinas from APP transgenic mouse strains contain 18–70 kDa proteolytic products from APP. The proportion of α-secretase generated C-terminal fragments in transgenic retinas was higher than the fragments generated from β-secretase. However, in ELISA assays, retinal Aβ1–42 was 75 times lower than in transgenic brains and remains undetectable by western blot, indicating that much less Aβ is generated in the retina compared with the brain (154). The age-dependent increase in plaques in the outer and inner plexiform layers (OPL/IPL), INL/ONL, and ganglion cell layer (GCL) (155) coincides only partly with the upregulation of APP, which is seen only in the RGC and INL regions (149). In line with these data, transgenic AD mice display both neuroinflammation and neurodegeneration mostly in the GCL (152, 156), where they correlate with APP induction and Aβ accumulation (149).

Interestingly, a recent study showed that amyloidopathy occurs in the retina prior to the brain in TgAPP/PS1 mice, suggesting that in AD patients, Aβ deposits may also be detected in the retina prior to the brain (10). The study of retinal amyloidopathy may be useful, not only to understand the molecular mechanisms involved in AD but also to search for early-stage AD-related biomarkers. This prospect is even more interesting, considering the possibility of developing a non-invasive method to diagnose early-stage AD through direct retinal imaging.

### *Synaptic Dysfunction in the Retina*

Available data concerning AD-related retinal synaptic dysfunctions come exclusively from electroretinogram (ERG) recordings, which give insight into the global electrical response of the retina to a light stimulus. ERGs performed in AD patients at the advanced stages of pathology revealed a significant reduction in the amplitudes of a- and b-waves as well as an increased latency of the response (156, 157). Analogous data have been reported in the aged TgAPP/PS1 mouse model (155). However, while ERG recordings provide a rough estimate of the AD-dependent impairments in glutamate-mediated excitatory neurotransmission in the retina, they do not decipher the underlying mechanisms. Cellular electrophysiology studies (field-recording, patch-clamp) are needed in order to precisely define the neurochemical type of synapses and neurons that are the main targets of Aβ.

### *Neuroinflammation in the Retina*

The accumulation of Aβ deposits with age in the retina of a transgenic mouse model of AD is accompanied by an increase in immunoreactivity for MCP-1 and F4/80, which suggests that resident microglia are activated, as well as an increase in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive profiles in the GCL (149). These results suggest that Aβ-induced neurodegeneration is associated with neuroinflammation (149).

The subretinal microinjection of Aβ yields an adaptive, local inflammatory response, which consists of altered expression patterns of cyclooxygenase-2 (COX-2), glutamine synthetase (GS), inwardly rectifying potassium (Kir) channel Kir4.1, and aquaporin (AQP)-4 water channels in retinal Müller glia cells and of AQP-1 in photoreceptors. Activation of the CCL2/CCR2 chemokine axis, along with microglia activation and migration, is also detectable in this paradigm, whereas its inhibition provides neuroprotection against the deleterious actions of Aβ (158). Moreover, Aβ triggers gliosis characterized by glial fibrillary acidic protein (GFAP), vimentin, and nestin upregulation in Müller cells (159). These alterations are similar to those seen during neuroinflammation in the brain.

The upregulation of GFAP was further confirmed after Aβ injection into the vitreous fluid (160) in both acute (48 h) and delayed (5 months) settings. Remarkably, this study demonstrated a concomitant and selective loss of parvalbumin-expressing neurons in the INL and, to a lower extent, in the GCL (160). The latter finding suggests that, as in the AD brain (161) and transgenic AD mouse models (162, 163), parvalbumin-expressing inhibitory neurons in the retina may be the most vulnerable to Aβ.

### *Mitochondrial Dysfunction and Oxidative Stress in the Retina*

The neuroinflammation triggered by subretinal injection of Aβ was accompanied by oxidative stress in the inner and outer retinal segments with an increase in highly reactive unsaturated aldehydes 4-hydroxy 2-non-enal (HNE) and acrolein as well as in 8-hydroxy-2′-deoxyguanosine (8-OHdG), a measure of oxidative damage to DNA (159), which culminated in photoreceptor cell death (158, 159). Accordingly, an inverse approach consisting of intravitreous injection of mitochondrial respiratory complex inhibitors confirmed that inhibition of mitochondrial function and associated oxidative stress resulted in increased APP processing and Aβ accumulation. The latter alterations were also found to be accompanied with GFAP upregulation and glial activation (44).

### *Amyloid Microangiopathy*

Amyloid-beta accumulation has been found in the retinal and choroidal vasculature of AD mouse models, suggesting that Aβ may be implicated in alterations in local blood flow (149). Moreover, retinal veins in AD patients are narrowed, and the retinal blood flow is decreased (164). Most importantly, a very large case-controlled study (213 AD patients and 294 cognitively normal controls) of retinal microvasculature networks reported a significant decrease in the branching pattern index (fractal dimension) of the retinal venular tree and arteriolar tortuosity in patients (165). Taken together, recent studies in the brain and retina point to similar alterations in the microvasculature in mouse models and AD patients. Furthermore, retinal microvasculature alterations, accessible to non-invasive imaging, may reflect those occurring in the brain. In line with this assumption, abnormal retinal blood flow has been correlated with degree of cognitive impairment (AD versus MCI versus control subjects), suggesting that blood flow abnormalities may precede AD-related neurodegeneration (166).

## Age-Related Macular Degeneration

Age-related macular degeneration is an age-related retinal degenerative disease that causes irreversible vision loss. It is estimated that up to 50 million people worldwide are affected by AMD, and in western countries 5–10% of individuals over 60 years of age suffer from this disorder (167). AMD is characterized by the build-up of drusen deposits between the Bruch's membrane (BM) and the RPE, which lead to RPE cell abnormalities, dysfunction of the choroidal blood–eye barrier, and photoreceptor death (168, 169). The most common form of AMD is dry AMD, characterized by thickening of the BM, formation of drusen deposits, and activation of the innate immune response (170). The dry form may progress into the exudative (or wet) form, which is characterized by choroidal neovascularization and retinal edema (171). In some cases, drusen deposits continue to expand and can coalesce, giving rise to the degeneration of a large area of RPE and photoreceptors in a process known as geographic atrophy. Drusen is extracellular deposits composed of different proteins, including Aβ and complement members (172). The mechanism leading to drusen formation is still unclear but may involve the accumulation of toxic by-products of the phototransduction cycle (173). These toxic by-products cause oxidative stress and inflammation, which play a central role in AMD progression (42, 174–177). Drusen-associated amyloidogenic proteins have recently been identified as oligomers (172).

Retinal cells that overlie both soft and hard drusen display numerous structural and molecular abnormalities. Normally detectable only in the outer segments of rod photoreceptors, rod opsin immunolabeling was also observed in the inner segment, cell body, axon, and axon terminal of photoreceptors that overlie drusen (178).

Similar to AD, the risk of developing AMD is also linked to some apolipoprotein E (APOE) polymorphisms. However, in contrast to AD, it has been shown that the e4 allele of the gene encoding APOE is associated with a lower risk of developing AMD, while the e2 allele is associated with a higher risk. Other polymorphisms associated with the development of AMD are linked to genes encoding components of the complement system (170). The polymorphism Y402H in complement factor H (CFH), for example, is the first genetic risk factor for both forms of AMD (179–181). It occurs in 33% of individuals and is associated with a 48% risk for developing AMD (182). CFH is the main inhibitor of the alternative pathway, a key component of the innate immune response. *cfh* KO mice also show features of AMD (183). The mechanisms by which CFH and polymorphisms in the gene affect AMD remain unknown. In 2016, the CFH Y402H polymorphism was identified as a risk factor for AD in a very large cohort of patients (184), confirming previous studies (185).

### Synaptic Dysfunctions

There is currently no data on putative synaptic dysfunctions in AMD. This may be related to the fact that the main target of neurodegeneration in AMD is the RPE, which is not part of the neuronal network *sensu stricto*. However, RPE cells are excitable, and it would be interesting to explore Aβ-related effects on their excitability.

Drusen-associated abnormalities in the synaptic terminals of photoreceptor neurons have been reported. In AMD-afflicted retinas, but not in normal aged human retinas, a large number of photoreceptor synapses across the entire retina retract into the ONL. This event evokes the subsequent outgrowth of dendrites from postsynaptic bipolar cells, again across the entire retina, and the subsequent rearrangement of synaptic contacts between the photoreceptor and bipolar cells. In addition, an increase in intermediate filament protein immunoreactivity (vimentin and GFAP) is observed within Müller glial cells in areas of the retina overlying drusen. However, other types of retinal neurons (i.e., bipolar, horizontal, amacrine, and ganglion cells) are all, at least structurally, unaffected (186).

### Mitochondrial Dysfunction and Oxidative Stress

In AMD, the accumulation of lipofuscin, i.e., cross-linked pigmentary deposits from photoreceptor membranes, favors RPE degeneration. Lipofuscin has damaging oxidant properties and has been associated with mitochondrial dysfunction. Similar to what happens within the brain, Aβ accumulation may further exacerbate this state of metabolic and oxidative stress (170). Analogously, Aβ accumulation may contribute to mitochondrial dysfunction in RGCs. Indeed, intracellular Aβ has also been observed in these cells, and it is likely that Aβ interferes with mitochondrial function, following the mechanisms characterized in AD (37).

### Neuroinflammation

Drusen formation leads to activation of the innate immune system and also to oxidative and metabolic stress, which progressively leads to neurodegeneration. Increased deposition of Aβ has been found in photoreceptor outer segments and in the membrane between the RPE and the BM, in the retinas of both aging humans and mice (51). It has been proposed that along with aging, gradual accumulation of debris may initiate the formation of drusen, which encapsulates different types of proteins, lipids, and inflammatory molecules (176). Among these proteins, extracellular Aβ derived from injured RPE may be included in drusen. Still, the role Aβ plays in this context is unclear. It has been shown that the oligomeric form of Aβ1–42 is implicated in the increased production of ROS, the alteration of RPE cell structure, and transepithelial permeability (91). In addition, Aβ may enhance the release of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor from RPE cells, favoring angiogenesis (187).

### Amyloid Microangiopathy

Amyloid microangiopathy has not been extensively studied in AMD. However, it has been proposed that microvascular leakiness may be caused by the promoting effect that amyloidogenesis may exert on neoangiogenesis. VEGF-mediated angiopathy plays a key role in choroidal neovascularization, which is a hallmark of exudative AMD (188). On the other hand, increased VEGF levels may be triggered by members of the complement system, such as C3a and C5a (189). It remains to be determined what triggers the activation of the complement system. Similar to what happens in AD, Aβ may promote its activation (190).

The activated complement system may in turn lead to increased vascular permeability and hypervascularization. This scenario has been experimentally verified in aged Tg2576 mice and postmortem AD brain tissue (191). Neovascularization is a major hallmark of exudative AMD, and by consequence, this form of AMD and AD may share pathological mechanisms in the context of blood–brain barrier impairments. However, a recent study (including 107 individuals diagnosed with AMD) reported no difference between venular and arteriolar calibers in the macula region, at least during the early stages of AMD (192) in agreement with a previous study (193).

## Glaucoma

Glaucoma is a progressive optic neuropathy that represents one of the leading causes of blindness worldwide. It is characterized by the loss of RGC neurons and their axons, with consequent structural changes in the optic nerve and visual field defects. The entire visual pathway, including intracranial optic nerve, lateral geniculate nucleus, and visual cortex, is affected (5, 194, 195). Therefore, glaucoma can be associated with other neurodegenerative disorders, such as AD, since the most vulnerable neuronal target (i.e., RGCs) is common for both pathologies.

One of the major risk factors for developing glaucoma is chronically elevated intraocular pressure (IOP). Accordingly, it has been shown that elevated IOP leads to ganglion cell changes that promote caspase activation and abnormal APP processing (196). Reducing IOP is the only therapy available to limit disease progression; however, the correlation between glaucoma and IOP has only been partially elucidated, and other factors clearly contribute to its pathogenesis (197, 198). Indeed, reducing IOP does not always stop disease progression (199), and some primary open-angle glaucoma patients show normal IOP (200).

It is presently unknown if Aβ is among the additional factors involved in the observed changes in IOP during glaucoma. Nevertheless, Aβ does appear to be a common denominator for glaucoma and AD. Indeed, in glaucoma patients, the level of Aβ in the vitreous fluid is decreased, while tau protein is increased (201). Similarly, in AD patients, the level of Aβ in the cerebrospinal fluid (CSF) is decreased, because of its reduced clearance, whereas tau protein is increased (202). In addition, increased levels of Aβ have been observed in RGCs in rat models of acute ocular hypertension (196, 203). Moreover, inhibiting Aβ production or improving its clearance reduced RGC death (203).

### Synaptic Dysfunction

Mechanisms of synaptic dysfunction in glaucoma have not yet been investigated.

### Mitochondrial Dysfunction and Oxidative Stress

Glaucoma has been shown to involve mitochondrial dysfunction (204), and oxidatively modified DNA, proteins, and lipids have been identified in affected patients (205). Importantly, the plasma level of F2-isoprostane lipid was correlated with heat shock protein 72 (HSP72) and heme-oxygenase-1, which are both known to be involved in the defense response against oxidative stress and are increased in glaucoma patients (206).

### Neuroinflammation

Transcripts of TNFα, IL-2, and IL-6 have been identified in the iris of neovascular glaucoma patients (207). The role of retinal glia-derived proinflammatory cytokines, notably IL-1β and TNFα, in glaucoma has been broadly recognized (208). Important insights into neuroinflammation-related mechanisms of glaucoma have been recently obtained in an elegant study using a rat model of glaucoma. The dominant-negative TNFα inhibitor, XPro1595, which selectively inhibits soluble TNFα, rescued Müller cell and microglia/macrophage activation after induction of ocular hypertension. Moreover, XPro1595 also prevented the TNFα-mediated induction of the $Ca^{2+}$-permeable GluR2 subunit of AMPA glutamate receptors, which are known to be causal in the cytotoxic effects of TNFα, as well as in the death of RGC neurons (209). These data formally demonstrate the causal link between neuroinflammation and neurodegeneration in glaucoma.

### Amyloid Microangiopathy

To date, putative Aβ-related structural and functional alterations of microvessels have not been investigated in glaucoma. Indeed, a host of publications (more than 2000 referenced in PubMed) deal with hemodynamic alterations that are consistently found in glaucoma (210). However, endothelin-1 and nitric oxide, known to be released by endothelial cells upon activation, are increased in open-angle glaucoma, suggesting the possible involvement of microvasculature in this pathology (210).

## CONCLUSION

Based on the evidence discussed in this review, it is increasingly clear that, at least in the case of Aβ-amyloidosis, the deleterious effects that Aβ exerts on both cerebral and retinal neurons are very similar. These similarities concern alterations at both the cellular and molecular levels, such as cytokine induction and mitochondrial failure, regardless of the particular disease. Furthermore, Aβ-related alterations, such as oxidative stress, microvasculature abnormalities, and neuroinflammation, are more related to amyloidosis than to the pathological context specific to each disorder (e.g., the different composition of Aβ plaques and drusen in AD and AMD).

Amyloid-beta may therefore be an attractive common target for immunotherapy in both AMD and AD. Encouraging results were obtained after administration of anti-Aβ antibodies in mouse models of AMD (211) and AD (212) that motivated human clinical trials, in spite of some secondary effects. Although the first-generation of Aβ vaccines in AD was interrupted because of severe cerebral hemorrhage (213), new molecules are currently in clinical trials. In particular, GSK933776 was effective in both AMD phase II (214) and AD phase I (215) trials. These clinical data further point to common mechanisms in AD and AMD. Consistently, treatment with an anti-Aβ antibody in a mouse model of AMD yielded a decrease in Aβ deposits both in the retina and the brain (211).

At this stage, many challenges remain for the future. For example, it is of utmost importance to determine whether a coincident oligopathy, such as the PD-associated α-synuclein amyloidosis, may affect Aβ-amyloidosis output in the retina. Understanding whether these two amyloidoses yield additive or synergistic pathological alterations may be very helpful for designing new and more global therapeutic approaches for all relevant diseases.

It is now largely recognized that neurodegenerative alterations in the retina reflect those occurring in the brain, thus raising the hope of using the retina as a source of diagnostic biomarkers for cerebral neurodegeneration. The retina has attracted much interest since, when compared with the brain, it displays the advantage of being relatively less complex structurally and more accessible to non-invasive exploration. Indeed, it may 1 day be possible to use the retina as a proxy to diagnose early neurodegenerative alterations in the brain to target them before neurodegeneration becomes irreversible.

## AUTHOR CONTRIBUTIONS

AM wrote the first draft of the manuscript, managed the references, and prepared the **Figure 2**. VD prepared the **Figure 1** and brought constructive changes to the text of the manuscript. CC significantly reviewed the text and worked on references indexing. FM made the major modifications in the course of successive reviewing. SK conceived and supervised the preparation of the review.

## FUNDING

The research leading to this review has received funding from the Fondation pour la Recherche Médicale FRM DVS20131228910 and the Service de Coopération et d'Action Culturelle du Consulat Général de France à Québec 65 502 (Coopération France-Québec) to SK.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AM and handling editor declared their shared affiliation, and the handling editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Neuroscience

REVIEW
published: 17 November 2016
doi: 10.3389/fnins.2016.00536

# The Eye As a Biomarker for Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in dementia and eventual death. It is the leading cause of dementia and the number of cases are projected to rise in the next few decades. Pathological hallmarks of AD include the presence of hyperphosphorylated tau and amyloid protein deposition. Currently, these pathological biomarkers are detected either through cerebrospinal fluid analysis, brain imaging or post-mortem. Though effective, these methods are not widely available due to issues such as the difficulty in acquiring samples, lack of infrastructure or high cost. Given that the eye possesses clear optics and shares many neural and vascular similarities to the brain, it offers a direct window to cerebral pathology. These unique characteristics lend itself to being a relatively inexpensive biomarker for AD which carries the potential for wide implementation. The development of ocular biomarkers can have far implications in the discovery of treatments which can improve the quality of lives of patients. In this review, we consider the current evidence for ocular biomarkers in AD and explore potential future avenues of research in this area.

**Keywords: biomarker, Alzheimer's disease, neurodegeneration, ocular, eye, retina**

OPEN ACCESS

***Edited by:***
*Raymond Chuen-Chung Chang, University of Hong Kong, Hong Kong*

***Reviewed by:***
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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience*

***Received:*** *19 September 2016*
***Accepted:*** *03 November 2016*
***Published:*** *17 November 2016*

***Citation:***
*Lim JKH, Li Q-X, He Z, Vingrys AJ, Wong VHY, Currier N, Mullen J, Bui BV and Nguyen CTO (2016) The Eye As a Biomarker for Alzheimer's Disease. Front. Neurosci. 10:536. doi: 10.3389/fnins.2016.00536*

## INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disease leading to severe cognitive loss and eventual death. Of the dementias, AD is the most common, accounting for between 60 and 70% of all dementias, depending on geography (WHO, 2012). Cohort studies around the world show that the number of people with AD increases with age, with roughly 1 in 5 people suffering from AD by the age of 85 (**Figure 1**).

The risk of developing the disease with age doubles every 5.9 years from 3.1 per 1000 persons aged 60–64 to 175 persons per 1000 at age 95+ (WHO, 2012), making age the strongest risk factor for AD. Characteristic to the disease is the profound atrophy of the brain accompanied by amyloid-beta (Aβ) plaques and the presence of tau neurofibrillary tangles (NFTs). Besides aging, genetics play a major role in the development of sporadic AD. Carriers of the apolipoprotein E (APOE) $\epsilon 4$ allele (13.7% of the world's population) face an increased risk of developing the disease (Farrer et al., 1997), depending on whether they have one or two copies of the $\epsilon 4$ allele. The estimated lifetime risk of developing the disease is higher in women with one (30%) or two (60%) copies of the $\epsilon 4$ allele, compared with men (23 and 51% respectively, Genin et al., 2011). Environmental factors for the disease (**Figure 2**) include diabetes mellitus, midlife hypertension, present smoking, depression, cognitive inactivity, physical inactivity, obesity, level of education, lack of social engagement (Flicker, 2010; Barnes and Yaffe, 2011). These modifiable factors (**Table 1**) carry varying degrees

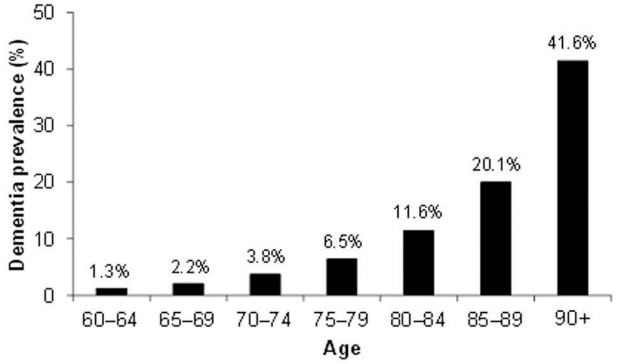


**FIGURE 1 | Dementia world prevalence by age.** Data based on meta-analyzed estimates of dementia prevalence (%) generated from Poisson random effects models from WHO (2012).

of risk and in combination may account for up to half of the AD cases worldwide (Barnes and Yaffe, 2011).

At present, physicians make the diagnosis of AD when patients already exhibit early cognitive losses. The diagnosis is formalized through mental state or cognitive examinations, alongside vascular and neurological assessments to rule out other causes (Burns and Iliffe, 2009). These tests signify the beginning of the irreversible process leading to dementia. The diagnosis is only confirmed *post mortem* though an examination of the brain. At present, the average survival from diagnosis to death is 4.6 years, affording little opportunity for treatment outside of palliative care. **Figure 3** summarizes the mechanisms leading to the formation of Aβ plaques and tau tangles, both of which are hallmarks of the condition.

Significant advances have been made in the development of *ante mortem* diagnostic tools or biomarkers for the disease. Biomarkers or "surrogate measures" of a disease are useful because they enable early diagnosis. Moreover, a good biomarker also enables assessment of drug efficacy both in the laboratory and in the clinic. Tools that can reliably triage drugs that are worth taking forward into progressively more expensive Phase I, II, and III clinical trial phases would considerably reduce the cost of drug development. Techniques that are simple, non-invasive, quantitative and objective lend themselves well to being biomarkers for preclinical and clinical trials. There is a growing need for a biomarker in Alzheimer's disease as recent clinical findings suggest that successful treatment needs to start in the prodromal stages of the disease (Ising et al., 2015). How such prodromal stages can be identified is thus of critical importance.

At present, the most well established biomarkers include those found in cerebrospinal fluid (CSF) (Aβ-42, T-tau, p-tau) and in the brain (fluorodeoxyglucose [FDG]- and Pittsburg Compound B [PiB]- Positron Emission Tomography (PET) with reported sensitivities and specificities of about 0.8 (Rabinovici et al., 2011; Ferreira et al., 2014). Whilst these methods have considerably advanced our understanding of the disease, clinically they fall short of the criteria necessary for large-scale population screening. Such methods can also be expensive, require repeat exposure to radiation (PET imaging) or are invasive (lumbar puncture to obtain CSF sample). The search for AD biomarkers has expanded to include other forms of brain imaging such as near infrared and brain volume scans (Hoffman et al., 2000; Csernansky et al., 2004; Klunk et al., 2004; Hintersteiner et al., 2005), as well as assays of, blood (Koyama et al., 2012), skin (Khan and Alkon, 2010; Khan et al., 2015), urine (Ghanbari et al., 1998), odor (Kimball et al., 2016), and olfactory deficits (Devanand et al., 2000; Tabert et al., 2005).

Given that many Alzheimer's sufferers report visual symptoms (Schlotterer et al., 1984; Sadun et al., 1987; Cronin-Golomb et al., 1993), there has been an increased interest in potential ocular biomarkers. Indeed, there have been reports that some visual symptoms can precede the onset of dementia, and have been attributed to the development of senile plaques and tangles in the visual regions of the brain (Mentis et al., 1996; McKee et al., 2006; Brewer and Barton, 2014). In addition, a visual variant of AD (VVAD) affecting relatively younger persons has been identified, though it is important to distinguish this as a separate pathophysiological entity known as posterior cortical atrophy. Patients suffering from VVAD typically first present with visual symptoms in the fifth or sixth decade of life and eventually the cognitive decline follows the course more typically seen in AD (Levine et al., 1993; Lee and Martin, 2004; Kaeser et al., 2015). In addition to potentially important early visual changes, the eye is very accessible and the retina can be easily imaged, thus making ocular biomarkers attractive. **Figure 4** summarizes the mechanisms thought to be involved in AD. Along this sequence of pathological changes are opportunities for various biomarkers including those involving the eye. These will be discussed in greater detail below.

## RETINAL BIOMARKERS

### Retinal Nerve Fiber Layer and Optic Nerve

The first histological evidence for retinal abnormalities in AD was presented by Hinton et al. (1986) who found widespread ganglion cell losses (the output neurons of the eye), retinal nerve fiber layer thinning (RNFL, ganglion cell axons) and optic nerve degeneration in AD versus healthy controls. This finding was supported by further studies (Blanks et al., 1996) and provides an anatomical basis for the use of retinal imaging as a potential marker of AD. Conventional fundus photography was met with limited success due to poor nerve fiber resolution (Tsai et al., 1991; Hedges et al., 1996).

The advent of commercially available, high resolution imaging modalities such as confocal scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) allow for repeatable *in vivo* quantification of optic nerve topography and nerve fiber layer. Scanning laser ophthalmoscopy, has the advantage over conventional photographs of the back of the eye in that it employs a confocal scanning laser system, thus allowing for fine depth resolution. Using SLO, Kergoat et al. (2001) and Danesh-Meyer et al. (2006) demonstrated RNFL thinning in the macula and peripapillary regions of the retina. Optical coherence tomography uses low coherence interferometry to detect small differences in *in vivo* refractive indices, thus returning high resolution cross sections of the retina. Using OCT, Berisha et al.

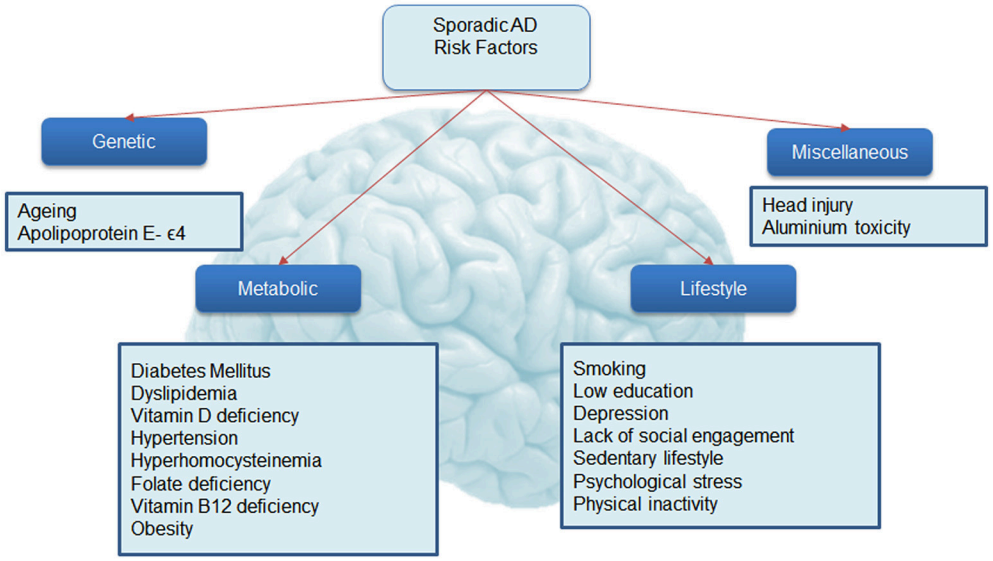


**FIGURE 2 | Risk factors associated with Alzheimer's disease.** This figure shows known associations with Alzheimer's disease.

**TABLE 1 | Risk factors for sporadic Alzheimer's disease.**

| Risk factor | Relative risk |
|---|---|
| Lack of social engagement | 2.34 (1.18–4.65) |
| Depression | 1.90 (1.55–2.33) |
| Physical inactivity | 1.82 (1.19–2.45) |
| Hypertension (midlife) | 1.61 (1.16–2.24) |
| Obesity (midlife) | 1.60 (1.34–1.92) |
| Smoking | 1.59 (1.15–2.20) |
| Low education | 1.59 (1.35–1.86) |
| Diabetes | 1.39 (1.17–1.66) |

*This table shows the most commonly identified modifiable risk factors in non-familial AD and associated relative risk.*

(2007) showed overall RNFL thinning in AD, with preference for superior RNFL loss. This finding is corroborated by a number of other studies (Lu et al., 2010; Kesler et al., 2011; Kirbas et al., 2013; Cheung et al., 2015). Cheung et al. (2015) in a large cohort of AD and patients with mild cognitive impairment (MCI) showed that the ganglion cell complex (which includes the RNFL, ganglion cell bodies and their dendrites) at the macula (where this anatomical feature has the best signal to noise characteristics) is a more sensitive indicator of the disease than RNFL thickness. Using this method, they were able to distinguish AD and MCI from healthy controls after adjusting for age, gender, ethnicity and OCT signal strength.

A challenge in using the macular ganglion cell complex or RNFL as biomarkers is its specificity, which is prone to confounds introduced by aging and other co-existing pathologies such as glaucoma. Perhaps one way to disambiguate age-related changes is to assess much wider areas of retina. Age-related changes are thought to be much more generalized, whereas there is some evidence for more sectoral losses in diseases such as AD (Parisi et al., 2001; Berisha et al., 2007; Kesler et al., 2011) and glaucoma. Whether AD and glaucoma exhibit mutually exclusive patterns of loss remains to be seen but the recent advent of wide field spectral domain OCT (Heidelberg Engineering, 2016) will increase our capacity to topographically map ganglion cell complex and RNFL changes.

As we learn more about the sequence of events and the neurodegenerative changes in AD there may be further opportunities for structural retinal biomarkers. For example, there is evidence that synapse loss predates neuronal loss and that the remaining neurons become less well connected to their synaptic partners. This may account for why synaptic density is the best correlate of cognitive decline in AD (DeKosky and Scheff, 1990; Scheff et al., 1990, 1993, 2007; Terry et al., 1991; Masliah et al., 1994; Ingelsson et al., 2004), which may also explain why the macular ganglion cell complex is more sensitive to MCI than RNFL thickness as it is inclusive of the inner plexiform layer containing dendrites of and synaptic connections between retinal ganglion cells and bipolar cells. In addition to synaptic loss, early change to neurons includes the disruption of microtubules (Matsuyama and Jarvik, 1989; Mandelkow et al., 2004; Gasparini et al., 2011). Ganglion cell axons in the RNFL contain microtubules, which give rise to the property of birefringence, whereby their refractive index depends on the polarization of the incident light. Thus, retinal imaging based on the projection of polarized light is extremely sensitive to the loss of microtubules in ganglion cells. Reductions in the birefringence signal as measured using scanning laser polarimetry (GDx) has been shown to precede RNFL thinning as measured by OCT (Huang et al., 2006; Fortune et al., 2008). Polarization sensitive OCT (PS-OCT) is a rapidly developing imaging modality that may have substantial implications for retinal imaging in AD (Pircher et al., 2011). By combining multiple techniques including wider topographical mapping, comparing inner plexiform and RNFL thickness and

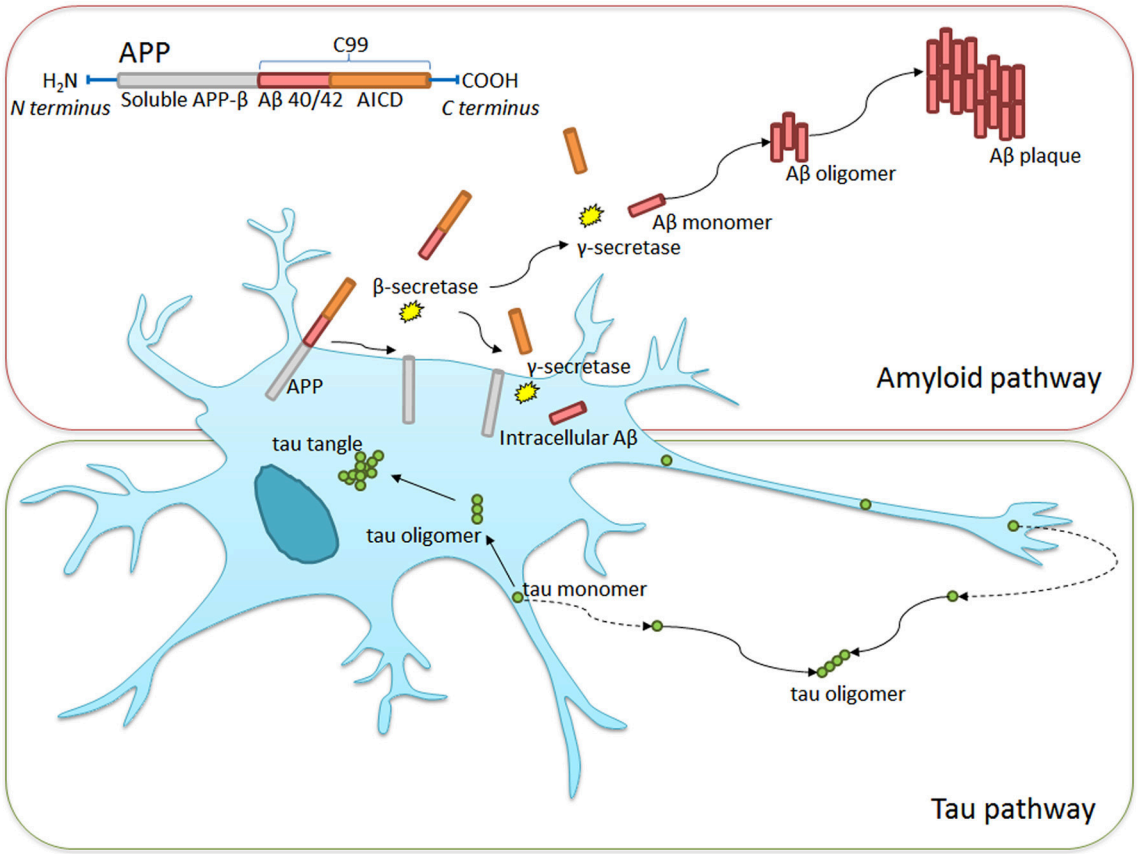


**FIGURE 3 | Intra- and extra-cellular Alzheimer's disease hallmark formation.** Amyloid precursor protein (APP) transmembrane protein contains between 365 and 770 amino acids beginning with the N- and ending with the C-terminus. β-secretase cleavage leads to the formation of a 99-chain amino acid at the C terminus (C99). It undergoes further cleavage via γ-secretase to form either Aβ-40 or Aβ-42 monomers. These monomers clump together, taking on complex formations eventually leading to Aβ plaque formation. Similarly, tau monomers clump to form complex oligomers and eventual neurofibrillary tangles, though this process is less well understood. Non-pathological APP processing via α-secretase is not shown in the diagram.

birefringence it may be possible to return certain signatures of loss for aging, glaucoma and AD thus improving specificity for retinal imaging biomarkers of AD.

## Retinal Blood Flow and Vasculature

The most commonly reported vascular problems in AD include impaired Aβ clearance, blood-brain-barrier compromise, reduced blood vessel density, smaller vessel diameter (vasoconstriction) and reduced blood flow (Zlokovic, 2011). Given similarities between blood vessels in the brain and eye (for review, see Patton et al., 2005), investigators have considered the potential usefulness of quantifying the blood vessels in the eye as a biomarker for AD. Berisha et al. (2007) in a small cohort showed a significant narrowing of retinal veins and reduced venous blood flow in AD compared with healthy controls using a laser Doppler imaging device. Using similar methodology, Feke et al. (2015) showed that differences in venous blood flow helped to distinguish MCI from AD and healthy control.

Larger population studies suggest that retinal vascular abnormalities are prevalent in AD patients. Using fundus photography and automated vessel segmentation software, Cheung et al. (2014) showed (1) a *reduction* in retinal vessel caliber; (2) a *reduction* in fractal dimension—a measure of the global branching pattern of the retinal vascular tree; (3) an *increase* in vessel tortuosity—a measure of the average degree of curliness/"non-straightness" of retinal vessels; and (4) *no change* to vessel branching angles–the angle subtended between two daughter vessels at each bifurcation. In addition to these parameters, Frost et al. (2013b) also reported that retinal vascular abnormalities, namely venular branching asymmetry and arteriolar length-to-diameter ratios were higher in healthy individuals with high plaque burden, as measured by PET imaging using PiB. Williams et al. (2015) (Williams et al., 2015) reported similar outcome, with the exception of changes in vascular caliber. The authors suggest that static retinal photographs may be more variable due to changes in vascular diameter with the cardiac cycle. Thus, vascular parameters that are robust to pulse driven variability may be useful for screening programs. A challenge in this regard will be whether AD related changes are tractable from age, atherosclerosis and vascular disease related abnormalities. Moreover, whether retinal vascular

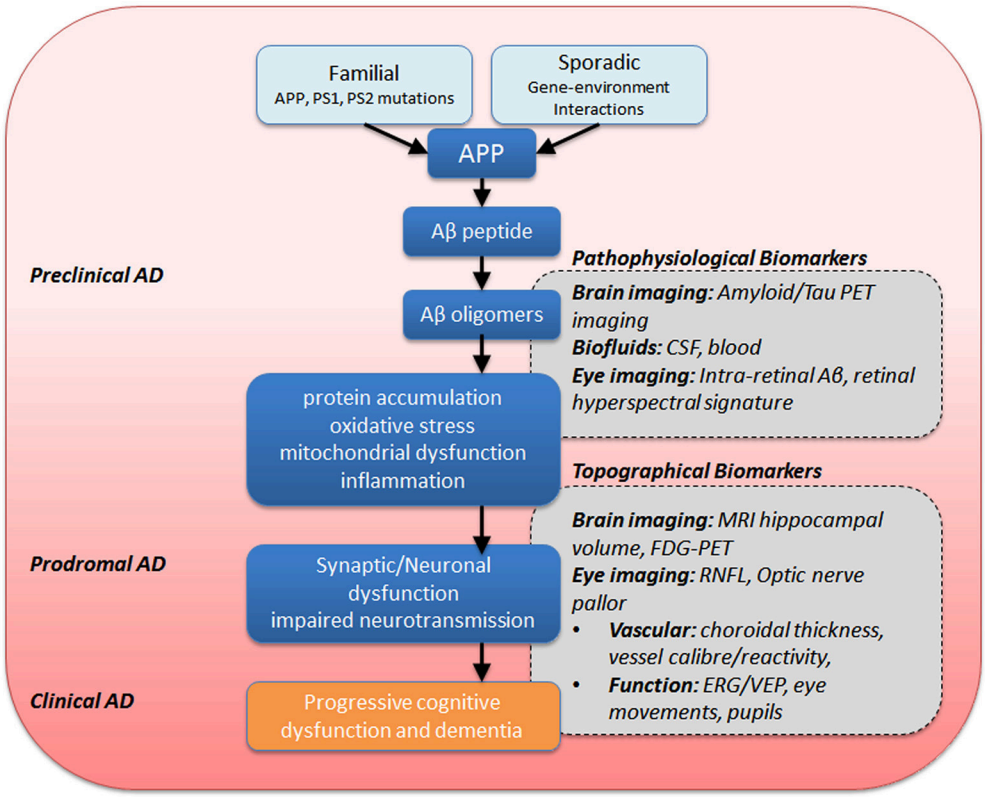


**FIGURE 4 | Summary of eye and brain biomarkers of AD.** APP, amyloid precursor protein; PS1/PS2, presenilin-1 −2, ERG, electroretinography; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PET, positron emission tomography; FDG-PET, fluorodeoxyglucose-PET; RNFL, retinal nerve fiber layer; VEP, visual evoked potential.

changes can differentiate mild cognitive impairment from AD is as yet unclear.

## Choroidal Thickness

Enhanced depth imaging (EDI) capabilities in modern OCT devices employing longer wavelengths in the range of 1060 nm (Wong et al., 2011) which allows for greater penetration into the deeper layers of the eye, allowing visualization of the choroid. The choroid which sits between the retina and the outer coat of the eye, accounts for the majority of the blood supply to the retina. A number of studies have assessed choroidal thickness changes in AD. Gharbiya et al. (2014) demonstrated that the choroid immediately below and within 1 mm around the fovea was ∼30% thinner in AD participants ($n = 21$) compared with healthy controls ($n = 21$). A 20% thinning of the choroid has also been reported by Bulut et al. (2016). Interestingly, Gharbiya et al. (2014) did not find reduced RNFL thickness. While this suggests that choroidal thinning preceded RNFL changes in AD, this should be interpreted with some care given the small sample size, potential noise generated from single-line manual segmentation of the choroid (Cho et al., 2014) and lack of control for signal strength (Ong et al., 2014).

Whilst better segmentation algorithms are already available to overcome the current limitations, the biggest challenge is that choroidal thinning undergoes significant diurnal fluctuations (Kinoshita et al., 2016) and its thinning is seen in many other conditions, including aging (Barteselli et al., 2012), myopia (Ho et al., 2013), uveitis (Baltmr et al., 2014), chronic obstructive pulmonary disease (Ozcimen et al., 2016). It also has limited application in the detection of age-related macular degeneration (Pilotto et al., 2015; Yiu et al., 2015) and glaucoma (Li et al., 2015; Toprak et al., 2016). Again, spatial mapping of choroidal thicknesses in relation to RNFL thinning may reveal specific areas of the eye that are more specific for Alzheimer's disease. This need for specificity underlies studies that have attempted to image hallmark AD deposits in the retina.

## Intraretinal Amyloid and Tau Deposition

In humans, Löffler et al. (1995) provided a histological demonstration that APP and Aβ accumulated (extracellularly, in sub-retinal deposits) in normal aged retinas. It was not known if any of these patients had AD prior to death. It has been known for more than a decade that Aβ is found in drusen deposits in eyes with age-related macular degeneration but not in normal retinas (Johnson et al., 2002; Dentchev et al., 2003; Anderson et al., 2004). More recent findings suggest that Aβ deposits might also occur inside retinal neurons.

Studies in animal models confirmed that APP is expressed by retinal ganglion cells, in the inner nuclear layer of the retina (cell bodies of bipolar, amacrine and horizontal cells) and in the retinal pigment epithelium. These cell classes also express β-secretase and are capable of producing Aβ (for review, see Ratnayaka et al., 2015). In genetically modified mice that have abnormalities in APP processing, Aβ accumulates sporadically within the RGC (Dutescu et al., 2009; Du et al., 2015), in the plexiform layers (Perez et al., 2009), in the nuclear layers (Shimazawa et al., 2008), in blood vessel walls (Liu et al., 2009), Bruch's membrane, outer segments of photoreceptors (Hoh et al., 2010) and within the retinal pigment epithelium (Park et al., 2014). In humans, Hoh et al. (2010) also reported that Aβ accumulated in photoreceptor outer segments in post-mortem retina of AD patients. Given that PET imaging detected amyloid deposition in the brain well before the onset of clinical symptoms in AD (Pike et al., 2007; Rowe et al., 2007), retinal imaging of *in vivo* Aβ accumulation is an exciting prospect.

### Tagging AD Deposits

Koronyo-Hamaoui et al. (2011) were first to demonstrate successful non-invasive *in vivo* visualization of curcumin bound flurorescent Aβ in the retinas of bitransgenic mice ($APP_{swe}/PS1_{\Delta E9}$), with mutations to genes that regulate APP and PS-1. They verified these findings using immunohistochemistry co-labeling via anti-Aβ specific antibodies and Thioflavin-S. By vaccinating with an altered myelin-derived peptide (MOG45D), which has been shown to reduce Aβ plaques in the brain (Koronyo-Hamaoui et al., 2009), the authors showed that retinal deposits behave in a similar way, with fewer and smaller Aβ plaques. Specific curcumin binding of Aβ has also been demonstrated in donor brains and retinas from AD patients. No such staining was seen in healthy controls of the same age. The Commonwealth Scientific and Industrial Research Organization (CSIRO, Australia) have shown promising preliminary data in humans employing curcumin contrast in patients confirmed to have deposits in the brain using PiB-PET imaging (Frost et al., 2014).

Though promising, other studies have not found the same outcome. Schön et al. (2012) in a small sample of 6 post-mortem retinas from confirmed AD patients failed to find Aβ plaques. They also report that they were unable to detect Aβ plaques *in vivo* using amyloid-binding fluorophores (BSc4090) in $APP_{swe}/PS1_{\Delta E9}$ mice. Schön et al. (2012) suggest that hyperphosphorylated tau could potentially be a better marker for the disease, having demonstrated its presence in the retina of the P301S human tau mouse line using *in vivo* scanning laser ophthalmoscopy imaging of (trans,trans)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB)-bound tau aggregates. These tau aggregates were also present in 5 out of 6 of the AD human retinas studied. However, Ho et al. (2014) failed to find either phospho-tau deposits or Aβ in the retina of 11 confirmed AD donors using standard histological techniques, including Aβ antibodies, thioflavin and congo red, that are well established for brain tissue. The authors conclude that AD hallmarks do not deposit in the eye in a manner analogous to the brain, though they concede that the use of different assays; limited retinal cross sections instead of wholemount; and paraffin embedded sections instead of frozen tissues; might limit the sensitivity in the detection of these proteins.

### Visualizing AD Deposits without Tagging

Newer imaging modalities that target specific reflectance characteristics of biological material hold the promise of being non-invasive, contrast agent free and potentially specific. One such method is hyperspectral imaging, whereby tissue reflectance to a wide range of incident wavelengths are quantified (More and Vince, 2015). Using this technique, More and Vince (2015) were able to determine that Aβ has a unique hyperspectral signature, capable of distinguishing these deposits in *ex vivo* preparations of brain and retina from a mouse model of AD. More recently, the authors applied this technology to a live mouse retina, demonstrating that the hyperspectral signatures are preserved even when imaged through the ocular media. This discovery provides the first evidence for the non-invasive *in vivo* detection of AD using hyperspectral imaging without the need for an extraneous agent (More et al., 2016).

Another emerging imaging technique is the use of cross-polarizers to distinguish *ex vivo* human donor retinas with AD from controls (Campbell et al., 2015; Hamel et al., 2016). This method capitalizes on the fibrillary arrangement of Aβ resulting in specific changes to birefringence that are quantifiable using Mueller matrix polarimetry. Along these lines, the advent of PS-OCT may also prove to be useful.

Direct visualization of AD hallmarks with or without a contrast enhancing agent in the retina may be considered the most promising biomarker due to its specificity for the disease. However, ongoing work is needed to verify that Aβ plaques or accumulations, are indeed present in human retinal tissues; and that such deposits indicate or are predictive of brain deposits.

## Electrophysiological Signature

Functional change in the electrical response of specific brain regions may be early markers in AD. This might arise as neuronal changes signaling damage which may precede the conversion to clinical disease (Alberdi et al., 2016). Not surprisingly, researchers have measured visually evoked potentials, using electrodes on scalp overlying the occipital region on either side of inion, as a way to differentiate AD and healthy control volunteers. A checkerboard stimulus that is reversing in contrast produces P1 and N1 components that are substantially reduced in patients with advanced AD (Parisi et al., 2001; Stothart et al., 2015). This is associated with losses in the retinal ganglion cell derived pattern electroretinogram, which is measured using electrodes on the eye in response to the same stimulus (Parisi et al., 2001; Stothart et al., 2015). Furthermore, studies using multifocal electroretinogram, which uses finer checks to allow spatial localization of responses to small patches of retina, have shown changes in the macular region in early AD patients (Moschos et al., 2012). Electroretinogram deficits have also been found in murine models of Aβ deposition (Perez et al., 2009; Krantic and Torriglia, 2014). More recently, Parthasarathy et al. (2015) and Gupta et al. (2016) showed an association between Aβ burden in the retina and inner retinal function; suggesting altered

**TABLE 2 | Ocular changes associated with Alzheimer's disease.**

| Structure | Biomarker | Highlights |
|---|---|---|
| Pupils | Pupil flash response[a] | High speed video pupillography<br>a. Reduced mean constriction velocity<br>• sensitivity 0.74, specificity 0.71, AUC 0.76<br>b. Reduced maximum constriction, AUC 1.0<br>c. Constriction amplitude decreased in AD with repeated flashing |
| Lens | Aβ accumulation in supranuclear layer[b] | Topical Aftobetin hydrochloride ointment with fluorescent ligand eye scanning system shows greater fluorescence in AD vs. HC.<br>• sensitivity 0.85, specificity 0.95, AUC 0.915 |
| Retina | Aβ hallmark detection[c] | a. Curcumin (injected and/or orally administered) binding to amyloid in mouse retina, fundus camera.<br>b. Hyperspectral analysis distinguishes normal from AD mice.<br>c. Amyloid fibrillary signature detected using Mueller matrix polarimetry. |
| | OCT[d] | a. Retinal nerve fiber layer thinning<br>• Superior RNFL, AUC 0.60<br>• Macula Ganglion Cell complex, AUC 0.66<br>b. Choroidal layer thinning using EDI-OCT |
| | Vascular changes[e] | a. Retinal venous blood flow reduction<br>b. Retinal vessel width reduction<br>c. Retinal vessel increased tortuosity<br>d. Retinal vessel increased branching complexity |
| | Electroretinogram Visual Evoked Potential[f] | e. Pattern Electroretinogram: Slower N35, P50 implicit time and reduced P50 and N95 amplitudes<br>f. Multifocal Electroretinogram: reduced P1 amplitudes<br>g. Pattern Visual Evoked Potential: Slower P100 implicit time |
| Optic Nerve | Pallor[g] | Optic disk color analyzed using Laguna Optic Nerve Hemoglobin software shows reduced optic nerve hemoglobin in AD. |

*This table shows a summary of key studies documenting ocular manifestations in Alzheimer's disease.*
[a] *Prettyman et al. (1997), Fotiou et al. (2000), Fotiou et al. (2007), Frost et al. (2013a), Bittner et al. (2014).*
[b] *Kerbage et al. (2013, 2015).*
[c] *Koronyo-Hamaoui et al. (2011), Campbell et al. (2015), More and Vince (2015).*
[d] *Parisi et al. (2001), Iseri et al. (2006), Berisha et al. (2007), Paquet et al. (2007), Lu et al. (2010), Kesler et al. (2011), Kirbas et al. (2013), Moreno-Ramos et al. (2013), Ascaso et al. (2014), Larrosa et al. (2014), Cheung et al. (2015).*
[e] *Berisha et al. (2007), de Jong et al. (2011), Cheung et al. (2014), Frost et al. (2013b), Feke et al. (2015), Williams et al. (2015).*
[f] *Parisi et al. (2001) Krasodomska et al. (2010), Sartucci et al. (2010), Moschos et al. (2012), Stothart et al. (2015)*
[g] *Tsai et al. (1991), Bambo et al. (2015).*

neurotransmission between photoreceptors and inner retinal cells. Whether such changes are specific to AD remains to be seen.

# NON-RETINAL BIOMARKERS

## Pupillary Reactions

Pupil size and the pupillary response to light is determined by the balance of forces exerted by the iris sphincter and dilator muscles. The former is influenced by cholinergic receptors found in the parasympathetic system originating from the Edinger-Westphal nucleus; whilst the latter is innervated by noradrenaline receptor based postganglionic sympathetic system arising from the superior cervical ganglion. It is well established in AD that selective nicotinic acetylcholine receptor loss accounts for symptoms closely associated with the severity of the disease (Buckingham et al., 2009). Indeed, four out of the five US Food and Drug Administration approved treatments for AD are cholinesterase inhibitors, which promote neurotransmission and masks some of the symptoms of AD.

Scinto et al. (1994) proposed the use of a clinical "pupil dilation test" to expose AD cholinergic dysfunction. Central cholinergic depletion results in upregulation of peripheral receptors and thus hypersensitivity to cholinergic agonists, or a reduced sensitivity to cholinergic antagonists. Using a dilute agonist such as pilocarpine 0.0625% (Idiaquez et al., 1994) or muscarinic antagonist such as tropicamide 0.01% induced an abnormally large pupillary constriction or dilation in AD patients, respectively. An alternative explanation was offered by Hou et al. (2006) who showed evidence that the locus coeruleus of the brain was responsible for adrenergic iris dilator input; which when damaged in AD resulted in less sympathetic monotone resistance to low dose tropicamide in AD. Recent studies have been unable to replicate these findings. In a review of more than 20 available studies on the subject, Frost et al. (2010) concluded that the reliability of a "pupil dilation test" for AD remained questionable.

Given that cholinergic neurotransmission is altered in AD, this might result in a change in the pupil's response to light. Indeed, a

decrease in pupil diameter response to abrupt changes in room illumination (Prettyman et al., 1997) or a single flash of light (known as the pupil flash response, PFR) has shown promise as a biomarker for AD (Fotiou et al., 2000). The latter study went on to show that the PFR parameter that best distinguished AD patients from healthy controls was the maximum constriction acceleration (Fotiou et al., 2007). Others have also linked PFR changes to amyloid plaque burden in the brain (Frost et al., 2013) and were also able to distinguish asymptomatic carriers of an amyloid beta gene mutation (APPGlu693Gln) from non-carriers (Frost et al., 2013). Whilst standard flash pupillometry was able to distinguish AD from healthy controls, only subtle timing differences were reported between groups.

Modifications are therefore being developed to enhance the sensitivity of this technique such as the use of chromatic light to target specific susceptible neurons in the retina, in particular the retinal ganglion cells (Rukmini et al., 2015). Another possibility is the use of a slower flash with an upwards "ramp" of increasing intensity may draw out more subtle differences in maximum constriction acceleration (Lei et al., 2014).

To this end, Bittner et al. (2014) suggest that repetitive flashes fatigue the system resulting in more pronounced PFR amplitude attenuation in AD and MCI patients compared with healthy controls, and correlated better with cognitive test scores (MMSE). They hypothesized that repeated light stimulation in AD patients challenges the interaction between a deficient sympathetic tone and parasympathetic overtone, resulting in smaller pupils and progressive loss of amplitude. Taken together, these studies show that pupil flash responses using high-speed video-pupillography might be a promising biomarker as it is directly assesses cholinergic integrity in the nervous system. It remains to be seen whether taking AD medications such as cholinesterase inhibitors blunt this effect. Certainly, more studies are required to establish the reliability and repeatability of this emerging technology.

## Imaging the Crystalline Lens

The role of the crystalline lens is to focus incident light from the outside world onto the retina. Primarily composed of crystallin proteins, the high protein concentration and clear optics presents an opportunity for researchers to study protein aggregation in the eye. Aβ has been shown to deposit in the crystalline lenses of monkeys, rats, rabbits (Frederikse et al., 1996) and transgenic mice (Frederikse and Ren, 2002). Goldstein et al. (2003) first reported the discovery of an AD specific cataract in humans, where they show Aβ deposits in the cytoplasm of the equatorial supranuclear/deep cortical lens fiber cells. This "AD cataract" does not impair vision and is difficult to detect in a routine eye examination unless a full dilation is achieved (Moncaster et al., 2010). Others were unable to detect Aβ in the lens using either standard immunohistochemistry methods (Michael et al., 2013; Ho et al., 2014) or confocal Raman spectroscopy (Michael et al., 2014). In a clinical study, Bei et al. (2015) did not find specific cataracts in a cohort of AD patients whose pathology have been confirmed using both PiB-PET imaging and CSF analysis.

Kerbage et al. (2013) suggested using a florescent amyloid binding ligand in order to maximize the chances of detecting Aβ in the lens. By compounding the ligand substance (aftobetin hydrochloride) into a sterile ophthalmic ointment suitable for topical application; in combination with an *in vivo* pulsed laser fluorescent spectroscopy in a group of 20 AD and 20 healthy controls the authors were able to detect supranuclear amyloid in the lens of most AD patients with a sensitivity of 85% and specificity of 95% (Kerbage et al., 2015). The authors found a correlation between fluorescence uptake values in the lens with amyloid burden in the brain detected quantified using PET.

Despite the promise of Kerbage et al.'s (2013) recent study as a whole, the literature indicates that the presence of Aβ in the human lens remains to be confirmed. Nevertheless, it appears that fluorescently labeling Aβ in the lens may provide improved detection over conventional lenticular assessment. Further studies are needed to establish this is the case in larger cohorts of patients. Furthermore, ongoing studies are needed to prove that the system is capable of not just detection but potentially be able to stratify patients based on disease severity. **Table 2** summarizes the key studies which have shown promise as utility of the eye as a biomarker for AD. **Figure 5** schematically highlights those sites in the eye which show evidence for changes with AD as well as those currently being further investigated.

## Eye Movements

It has been recognized that patients with AD suffer difficulty with reading. This is in part due to suboptimal eye movements, which has been suggested to be linked to memory function (Fernández et al., 2014). It has been shown that AD sufferers present with increased latency when initializing voluntary eye movements, show decreased eye movement velocity, fail to fixate on a target or move in the wrong direction entirely and also fail to follow a moving target (Molitor et al., 2015). These fixation and movement errors reflect damage to their neural generators within the cortex and the brainstem. Although researchers have yet to find a distinct AD signature, eye movements show potential as a biomarker. Further studies are needed to establish a standardized test battery specific to AD and to demonstrate the ability to correlate oculomotor function with disease progression.

# POTENTIAL FUTURE BIOMARKERS

## Corneal Nerve Imaging

Corneal nerves are stratified into 3 layers and may be found in between the basal epithelium and bowman's layers (sub-basal nerve plexus), in between the bowman's layer and the anterior stroma (sub-epithelial nerve plexus) and within the stroma (stromal nerve plexus). Corneal nerve imaging has clinical utility post-corneal surgery as well as in diseased state such as keratoconus (a corneal ectasia), dry eyes and in diabetes (Patel and McGhee, 2009). Nerve growth factor is essential for the development and maintenance of neurons in the peripheral nervous system and for the integrity of cholinergic neurons in the central nervous system and in the cornea. As impaired nerve growth factor retrograde transport has been linked to cholinergic neurodegeneration in AD (Aloe et al., 2012) corneal nerve integrity might prove useful as a biomarker for the disease. Preliminary steps are being taken to be able to image

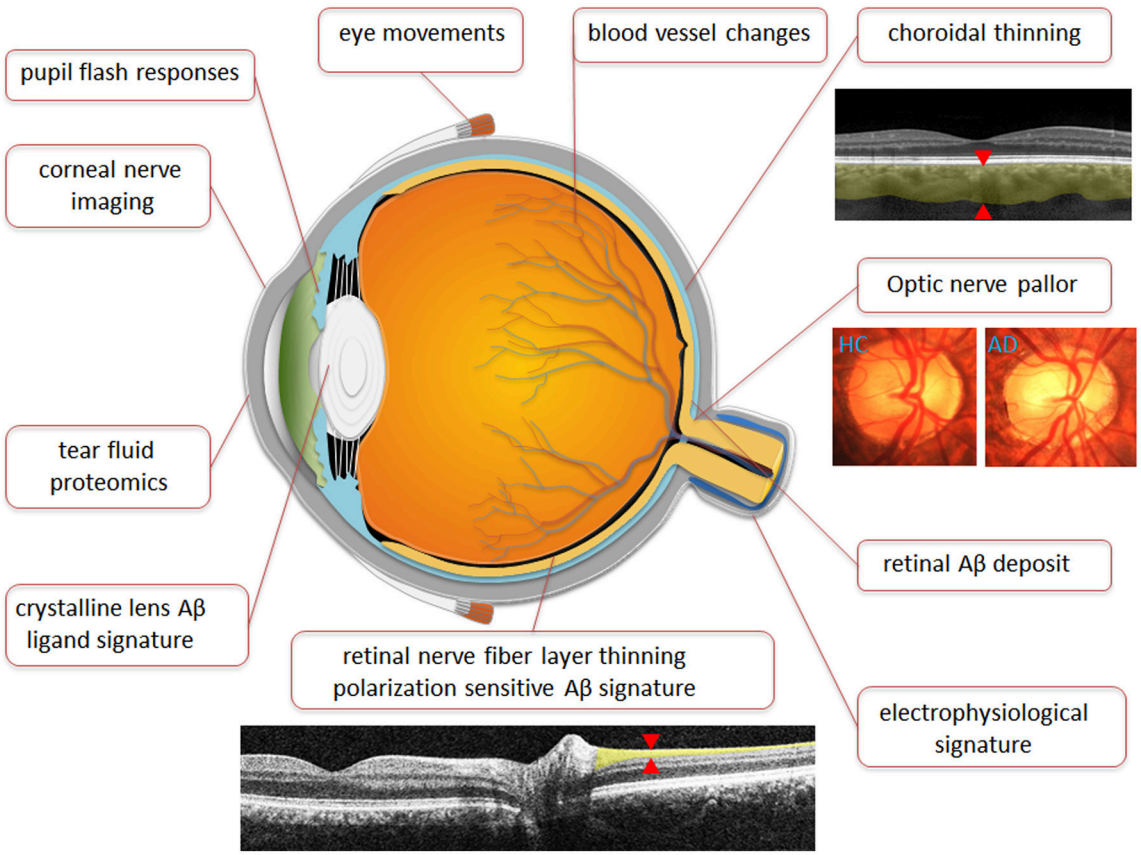


**FIGURE 5 | Anatomical representation of Ocular biomarkers.** Figure illustrates ocular biomarkers which have shown changes in AD patients as well as potential future ocular biomarkers as marked by location in the eye. Images are broadly representative of the techniques employed in studies. Red arrows denote where measurements are typically taken.

axonal transport *in vivo* (Abbott et al., 2013; Takihara et al., 2015). As corneal nerves can be imaged directly, a functional measure of axonal transport may prove to be more specific for AD.

## Ocular Fluid Biomarkers

Proteomics have been increasingly used since the 1990's for large scale analysis of proteins in health and diseases, such as AD (Butterfield, 2004). Proteomic analyses of aqueous humor samples have revealed the presence of AD related peptides. Tear fluid, being easy to access has also shown promise in the detection of neurodegenerative diseases such as glaucoma (Tezel, 2013) and was recently shown to be clinically viable (Kalló et al., 2016).

Micro RNA (miRNA) has gained significant attention as a class of non-coding regulatory RNA molecules capable of modifying gene expression at the post-transcriptional level by binding to the un-translated region of their target mRNAs. Several candidate miRNA namely hsa-miR-106, −153, −101, −29, −107 have already been shown to be implicated in regulating amyloid production (Kumar et al., 2013). That miRNA molecules have been isolated in tears warrants further studies as to their feasibility as a fluid biomarker for AD.

## SUMMARY

Only five treatments are currently approved by the United States Food and Drug Administration for AD, namely, rivastigmine, galantamine, tacrine, donepril and memantine. These are all cholinesterase inhibitors with the exception of memantine, which is an NMDA (N-methyl-D-aspartate) receptor antagonist. These medications delay the worsening of the symptoms in AD for some 6 to 12 months and work in about half of patients (Casey et al., 2010). As yet there is no approved treatment targeting the pathophysiological mechanisms underlying AD. Current candidate AD drugs include those that target either Aβ, APP, or tau metabolism by preventing oligomer efflux, modulating enzyme secretases, prevent aggregation, facilitate clearance and vaccination induced immunological clearance (Kumar et al., 2015). To test such drugs reliable and sensitive laboratory and clinical readouts are needed.

The discovery of AD biomarkers such as PET imaging and CSF molecules have considerably advanced our understanding of the disease. These biomarkers are crucial for disease monitoring and the recruitment of preclinical AD patients for clinical trials. Despite the success of these established biomarkers, their widespread implementation remains a challenge. Ocular

biomarkers for AD are still in their infancy and are not without their limitations. Many of the potential biomarkers discussed share substantial overlap with ocular and systemic diseases. Currently, the ocular biomarkers holding the most promise are those specific for AD pathophysiological such as the detection of Aβ-related retina changes. Additionally or alternatively, a battery of clinical functional and structural ocular assessments may improve specificity for AD. Eye care practices are well positioned to provide these technologies to the public. Already, sensitive imaging modalities such as fundus cameras and OCT are commonplace in eye clinics. Emerging technologies such as OCT angiography (using OCT to resolve the smallest blood vessels), EDI-OCT and PS-OCT will contribute to the increase in sensitivity and diagnostic capacity for the disease. With advances in this area, primary eye care practitioners may play a larger role in the provision of care for patients with Alzheimer's disease. A range of neurological diseases including AD, Parkinson's disease, frontotemporal dementia, vascular dementia, Neimann-Pick disease may stand to benefit from ocular biomarker technology, as a means to improve understanding, monitoring and to help facilitate of discovery of therapies.

## AUTHOR CONTRIBUTIONS

JL, BB, CN have made the following author contributions: Substantial contributions to the conception or design of the work; Drafting the work and revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. QL, ZH, AV, VW, NC, JM have made the following author contributions: Substantial contributions to the conception or design of the work; and Revising the work critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## FUNDING

CN, BB, AV, JM, (all authors), Samantha Budd Haeberlein, are joint investigators on an Australian Research Council Linkage grant LP160100126. BB is a sole investigator on Australian Research Council Linkage grant FT130100338.

## ACKNOWLEDGMENTS

The authors would like to thank Samantha Budd Haeberlein and Richard Hargreaves (Biogen Inc., 300 Binney St, Building 9, Sixth Floor, Cambridge, MA 02142) for their helpful comments and input in the preparation of this manuscript.

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**Conflict of Interest Statement:** CN, BB, AV and JM are joint investigators on an Australian Research Council Linkage grant LP160100126. NC is an employer of Biogen Inc. JM is an employee of AstraZeneca Neuroscience.

All other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 04 May 2017
doi: 10.3389/fneur.2017.00162

# Retinal Ganglion Cells and Circadian Rhythms in Alzheimer's Disease, Parkinson's Disease, and Beyond

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There is increasing awareness on the role played by circadian rhythm abnormalities in neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). The characterization of the circadian dysfunction parallels the mounting evidence that the hallmarks of neurodegeneration also affect the retina and frequently lead to loss of retinal ganglion cells (RGCs) and to different degrees of optic neuropathy. In the RGC population, there is the subgroup of cells intrinsically photosensitive and expressing the photopigment melanopsin [melanopsin-containing retinal ganglion cells (mRGCs)], which are now well known to drive the entrainment of circadian rhythms to the light–dark cycles. Thus, the correlation between the pathological changes affecting the retina and mRGCs with the circadian imbalance in these neurodegenerative diseases is now clearly emerging, pointing to the possibility that these patients might be amenable to and benefit from light therapy. Currently, this connection is better established for AD and PD, but the same scenario may apply to other neurodegenerative disorders, such as Huntington's disease. This review highlights similarities and differences in the retinal/circadian rhythm axis in these neurodegenerative diseases posing a working frame for future studies.

**Keywords: optic nerve, retinal ganglion cells, melanopsin, circadian rhythms, Parkinson's disease, Alzheimer's disease, Huntington's disease**

OPEN ACCESS

***Edited by:***
*Ivan Bodis-Wollner, SUNY Downstate Medical Center, USA*

***Reviewed by:***
*Rafael Linden, Federal University of Rio de Janeiro, Brazil*
*Weien Yuan, Shanghai Jiao Tong University, China*

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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *23 January 2017*
***Accepted:*** *07 April 2017*
***Published:*** *04 May 2017*

***Citation:***
*La Morgia C, Ross-Cisneros FN, Sadun AA and Carelli V (2017) Retinal Ganglion Cells and Circadian Rhythms in Alzheimer's Disease, Parkinson's Disease, and Beyond. Front. Neurol. 8:162. doi: 10.3389/fneur.2017.00162*

## INTRODUCTION

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most frequent age-related neurodegenerative disorders with an increasing prevalence with age (1, 2). They are both characterized by the frequent occurrence of sleep problems and circadian rhythm dysfunction (3–6). In the last decade, the role of the eye in influencing and regulating circadian rhythms has been clarified, starting from the discovery of the intrinsically photosensitive melanopsin-containing retinal ganglion cells (mRGCs) (7, 8). These cells constitute a small subset of regular retinal ganglion cells (RGCs) consisting of about 1–2% of the total, and they give origin to the retinohypothalamic tract through which they project to the suprachiasmatic nucleus (SCN) of the hypothalamus synchronizing circadian rhythms to the light–dark cycle (9). Besides this predominant function, they also play an important role in many non-visual functions of the eye, regulating sleep through the connections with the ventrolateral preoptic nucleus (VLPO) and the lateral hypothalamus (LH), melatonin secretion, and its suppression through the connections with the pineal gland, pupillary reflex through the olivary

pretectal nucleus, and also visual functions through the projection to the lateral geniculate nucleus (10–12).

In this review, the likely influence of the mRGC system in the pathogenesis of circadian misalignment in AD and PD is discussed, highlighting similarities and differences, starting from the observation that in both diseases, loss of regular RGCs has been documented by both histological and optical coherence tomography (OCT) studies, thus suggesting that the retina is actively and primarily involved in the neurodegenerative process characterizing both disorders. In fact, many studies describe optic neuropathies associated with AD and PD, which, however, display different patterns of RGC and axonal loss, possibly reflecting different pathogenic mechanisms. We here explore the connection between the eye and circadian functions and dysfunctions in AD and PD with particular reference to the mRGC system and its contribution to circadian functions.

## EVIDENCE OF INNER RETINA PATHOLOGY IN AD AND PD

### Alzheimer's Disease

Histological and OCT studies in AD demonstrated a significant loss of RGCs and consequent axonal depletion in the optic nerve. Hinton and colleagues in 1986 reported the first histological demonstration of optic neuropathy in AD describing loss of RGCs and axons in the optic nerve (13). After this seminal work, other histological studies reported degeneration of the inner retina in AD, more pronounced in the superior and inferior sectors of the optic nerve (14–20).

These histological findings are corroborated by many recent OCT studies pointing to retinal nerve fiber layer (RNFL) thinning in AD, as confirmed by a recent meta-analysis of 11 OCT studies in AD (21). RNFL thinning is more pronounced in the superior sector of the optic nerve (20–23) and is age related (20) (**Figure 1**). This pattern of RGC loss is consistent with the predominant inferior visual field defect described in AD patients (24). Moreover, a recent OCT study using segmentation analysis in a large series of AD patients showed a significantly reduced macular retinal ganglion cell–inner plexiform layer thickness in AD retinas compared to controls (25).

The pattern of axonal loss in the optic nerve, for example, the prominent superior quadrant involvement, is consistent with the histological findings that magnocellular RGCs are more vulnerable to AD pathology (16), which also resembles the pattern of RGC loss described in glaucoma (26). Recently developed *in vivo* imaging methods, such as the detection of retinal cells undergoing apoptosis (DARC), are extremely promising in quantifying and visualizing *in vivo* RGC loss in AD retinas (27).

The presence of the cerebral hallmarks of AD, such as amyloid plaques, in the retina gives strength to the specific vulnerability of the eye, and in particular of the inner retina to AD pathology. Koronyo-Hamaoui and colleagues provided the first demonstration of extra-cerebral Aβ deposits in postmortem human flat-mounted retinas of AD patients and *ex vivo* in APPSWE/PS1ΔE9 transgenic mice after curcumin administration (28). Subsequent studies confirmed the presence of specific amyloid pathology, including both extracellular plaques and intracellular Aβ deposits, more evident in the superior quadrant, and increased Aβ peptides levels in human AD retinas (20, 29, 30) (**Figure 1**). Other promising imaging techniques, recently developed for visualizing amyloid deposits in AD retinas, include hyperspectral imaging (31), the use of cross-polarizers (32), and the polarization-sensitive OCT (33). Schön and coauthors also demonstrated the presence of the other hallmark of AD pathology, such as the phosphorylated tau, in human AD retinas (34).

Finally, our group recently demonstrated that a specific subpopulation of RGCs, the mRGCs, are specifically lost in AD and affected by the amyloid pathology. In fact, using melanopsin and Aβ co-staining, our group showed that Aβ deposits are evident within and around these cells affecting also mRGC neuritis (20). Remarkably, the loss of these cells is evident even with a normal RGC count, pointing to a specific AD pathology affecting mRGCs (20). The loss of these cells is particularly relevant for interpreting the occurrence of sleep and circadian disturbances in AD (see next section).

### Parkinson's Disease

The occurrence of visual problems is a frequent finding in PD patients. These include blink, dry eyes, reduced visual acuity, contrast sensitivity, color vision abnormalities, oculomotor disturbances, and visual hallucinations (35, 36).

In particular, contrast sensitivity abnormalities are related to dopamine depletion at the retina levels (37–39) and can be partially reversed by the administration of L-DOPA therapy in PD patients (40). In fact, dopaminergic amacrine cells in the retina regulate the center-surround organization of RGC receptive fields and their dysfunction leads the retina to be in an inappropriately dark-adapted state (35). Color vision in PD patients is an early sign involving, at difference with the color defects observed with aging, the protan–deutan axis (red–green) (41). Interestingly, color vision abnormalities have good discriminative power in distinguishing PD patients from controls in the early stage of disease and may predict the conversion of idiopathic REM behavior disorder patients to PD (42, 43). However, the Farnsworth–Munsell 100 Hue test, commonly used for testing color abilities in PD, is influenced also by cognitive functions such as executive functions, and this must be taken into account in the interpretation of these results.

Besides the retinal dopaminergic depletion, which explains the occurrence of contrast sensitivity abnormalities in PD, there are multiple evidences pointing to RGC loss in PD (44, 45) (**Figure 1**). The presence of optic neuropathy has been reported by many OCT studies and, interestingly, the pattern of axonal loss resembles that typically seen in mitochondrial optic neuropathies, affecting the temporal sector of the optic nerve, i.e., the papillomacular bundle (RGC) (44, 46, 47). This pattern of RGC loss, which affects predominantly the parvocellular component, is clearly distinguishable from that described in AD, for which more frequently the magnocellular RGCs are affected (16, 20, 21) and other Parkinsonian syndromes, such as multiple system atrophy (**Figure 1**) (48).

Moreover, the optic neuropathy in PD is more pronounced in the eye contralateral to the most affected body side (46), suggesting a congruent asymmetry of the neurodegenerative process affecting

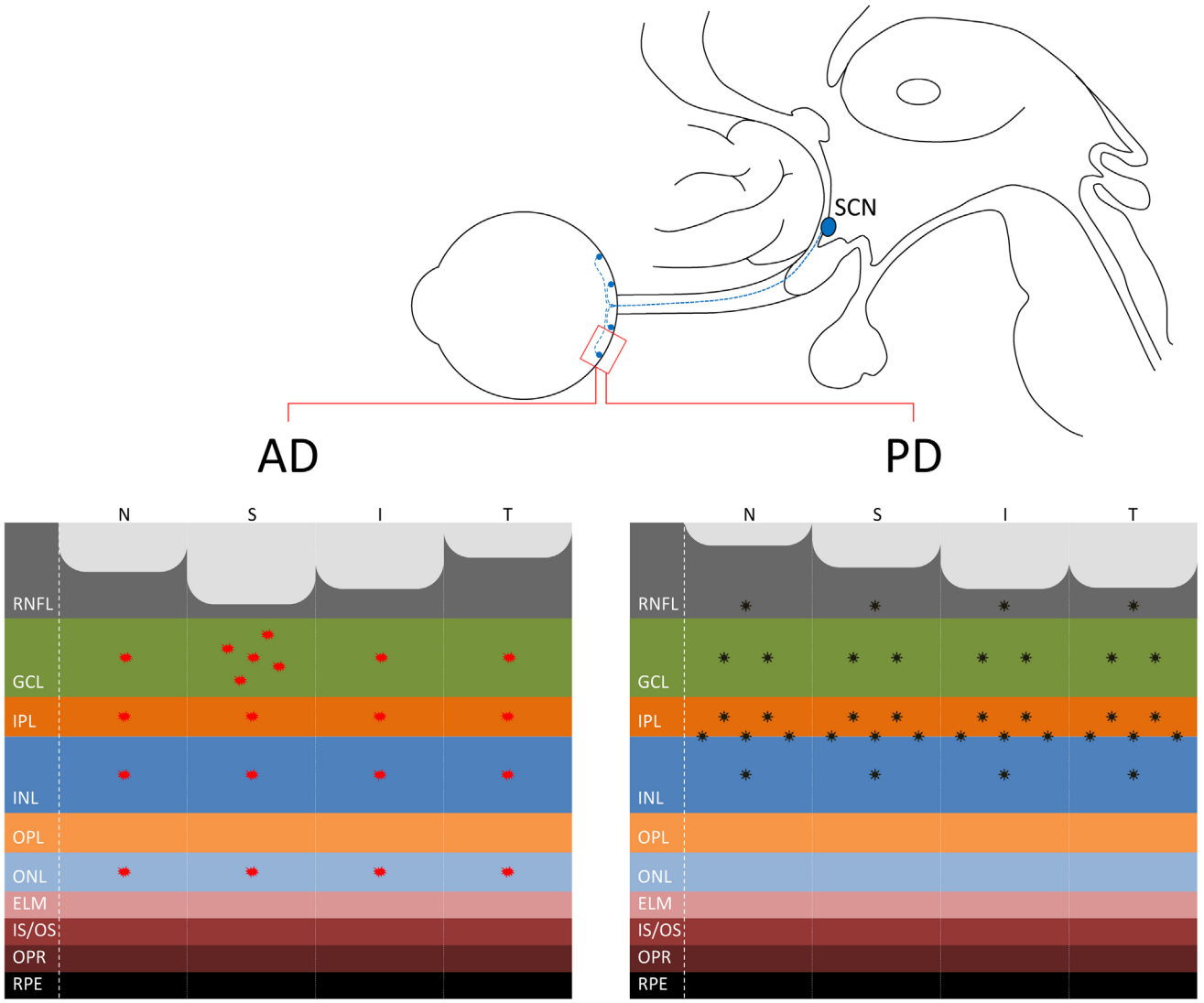


**FIGURE 1** | **(Upper panel)** The connection between the eye and the suprachiasmatic nucleus (SCN) of the hypothalamus through the retinohypothalamic tract, originating in the retina from melanopsin-containing retinal ganglion cells (mRGCs) (in blue), is shown. **(Lower panel)** At the retina level, where are located also the mRGCs, the distinct pattern of axonal loss [retinal nerve fiber layer (RNFL) thinning] demonstrated by optical coherence tomography studies is reported for Alzheimer's disease (AD) (21) with a more pronounced loss in the superior quadrant (left) and Parkinson's disease (PD) with a more evident loss in the infero-temporal quadrants of the optic nerve (44, 46, 49) (right). Moreover, the figure depicts the pattern of β-amyloid deposition in AD, more evident in the superior quadrant and ganglion cell layer (GCL) [for a review, see Ref. (30)], and α-synuclein in PD in the inner retina and in particular at the inner plexiform layer (IPL)–inner nuclear layer (INL) interface (51, 52).

also the retina. This asymmetry has been also documented for the foveal remodeling demonstrated in PD patients, as a hallmark of retinal pathology in PD (49, 50).

Finally, recent studies reported the presence of α-synuclein deposition in the retina of PD patients and in particular in the inner retina, pointing to a specific PD pathology affecting the eye (51, 52) (**Figure 1**). Interestingly, the staining of phosphorylated synuclein (p-syn) shown by Beach and colleagues (52) affected a large cell with an extensive dendritic tree, which resembles an mRGC. The possible occurrence of α-synuclein pathology affecting the mRGCs in PD may contribute to the occurrence of circadian dysfunction in PD that remains to be tested.

## CIRCADIAN RHYTHM DYSFUNCTION IN AD AND PD

### Alzheimer's Disease

Sleep and circadian disturbances are a frequent complaint of AD patients, appearing in the majority of cases early in the disease course and including daytime somnolence, increased sleep latency, and frequent night-time awakenings with sleep fragmentation (3). Some of the sleep changes described in AD are the same reported with aging, such as the reduction of slow wave sleep and the difficulty in sleep maintenance (53). Other sleep abnormalities, such as the reduction of REM sleep, are more specifically related to AD (54).

Circadian rhythm abnormalities are reported in AD patients even in the early stage of the disease including a delayed phase of the temperature rhythm (55), sundowning, i.e., the appearance of behavioral agitation in the evening (56), the reduction of melatonin levels during the night (57), and the circadian expression profile of clock genes (57, 58). Moreover, abnormalities of the rest–activity circadian rhythm, including increased intra-daily variability (IV) and reduced inter-daily stability (IS) and relative amplitude of the rest–activity rhythm are described in AD (59, 60), and the presence of circadian dysfunction may predict a poor outcome in terms of cognitive functions (59).

We recently demonstrated the presence of variable degrees of rest–activity circadian dysfunction in mild–moderate AD patients and a specific loss of mRGCs in AD retinas (20). A specific AD pathology affecting these cells may contribute to circadian dysfunction in AD patients. Moreover, these cells have a direct effect on sleep through direct and indirect projections to brain nuclei relevant for sleep regulation such as the LH and the VLPO (61, 62). The role of mRGC loss in contributing to sleep and circadian misalignment in AD is particularly relevant for its potential therapeutic options. In fact, the use of bright light therapy has been proven to be effective in counteracting sleep and circadian dysfunction in AD (63, 64).

Other possible components of the circadian imbalance demonstrated in AD is the presence of SCN neuronal loss and amyloid pathology documented in neuropathological studies of AD postmortem brain (65, 66), which correlates with the degree of rest–activity disruption (67) and can contribute secondarily to the mRGC loss demonstrated in AD retinas.

The strict relationship between cognition, sleep, and circadian rhythms is highlighted also by the demonstration that the presence of circadian dysfunction may predict the onset of dementia (68), as well as that sleep loss may promote the accumulation of amyloid and predispose to AD (53, 69). Many recent studies point to direct and indirect effects of circadian derangement in cognitive disturbances and hence dementia. Counteracting the circadian imbalance may have important clinical implications. A summary of circadian abnormalities in AD is reported in **Table 1**.

**TABLE 1 | Summary of circadian rhythm abnormalities in AD, PD, and HD.**

| | Circadian rhythm abnormalities | Reference |
|---|---|---|
| AD | • Daytime somnolence, increased sleep latency, and night-time awakenings | (3) |
| | • Delayed phase of temperature circadian rhythm | (55) |
| | • Sundowning | (56) |
| | • Reduction of night-time melatonin levels | (57) |
| | • Abnormal circadian expression profile of clock genes | (57, 58) |
| | • Increased IV, reduced IS, and reduced RA of rest–activity circadian rhythm | (20, 59, 60, 67) |
| PD | • Abnormal melatonin circadian rhythm (phase advance and decreased amplitude) | (70–73) |
| | • Increased IV, reduced IS, and reduced RA of rest–activity circadian rhythm | (74–76) |
| | • Reversal of circadian BP rhythm and loss of HR variability | (77, 78) |
| | • Abnormal temperature and cortisol circadian rhythm | (73, 80) |
| | • Abnormal peripheral clock genes circadian rhythm | (73, 81) |
| HD | • Delayed phase of the rest–activity rhythm | (88) |
| | • Abnormal melatonin circadian rhythm | (89) |
| | • Sleep fragmentation with night-time awakenings and reduced sleep efficiency | (90, 91) |

*AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; IV, intra-daily variability; IS, inter-daily stability; RA, relative amplitude; BP, blood pressure; HR, heart rate.*

## Parkinson's Disease

Sleep disturbances are reported in about 80% of PD patients (5). Furthermore, circadian dysfunction has been extensively described in PD patients, in terms of the following:

(1) Abnormal melatonin rhythm, i.e., abnormal phase angle of melatonin rhythm (70, 71) and decreased amplitude (72, 73). However, the phase advance of the melatonin rhythm documented by these studies was evident only in L-DOPA-treated PD patients, suggesting a possible influence of medications on these findings.
(2) Abnormal rest–activity rhythm, and in particular increased IV, reduced IS, and flattening of daily activity (74–76). However, a relevant influence of medications, motor, and non-motor symptoms (in particular cognitive disturbances and hallucinations) has been postulated also for these findings (6).
(3) Abnormal blood pressure (BP) and heart rate (HR) rhythm abnormalities such as reversal of circadian BP rhythm and loss of circadian HR variability (77, 78). However, these abnormalities can be influenced also by neurodegenerative changes of the autonomic nervous system documented in PD (79).
(4) Abnormal temperature (80) and cortisol rhythm (73).
(5) Abnormal clock gene rhythmicity in peripheral blood cells (73, 81).
(6) Circadian fluctuations of motor symptoms (82) with a worsening of motor functions possibly related to the dopamine level variations over the day.

Interestingly, at difference with AD, where neuropathological hallmarks of pathology such as amyloid plaques and neurofibrillary tangles are described in the SCN of the hypothalamus, the neurodegenerative changes characteristics of PD, such as synuclein deposition and Lewy bodies, are not reported in the SCN. This suggests that in PD, the circadian imbalance, at least in the early phase, is not primarily due to a master clock pathology (6). This is consistent with the finding that PD patients in the early stage of the disease do not exhibit frank circadian rhythm abnormalities, such as for melatonin and other hormones (6, 83). It is not clear, based on the currently available evidences, if circadian misalignment occurs as an independent hallmark of PD pathology or can be interpreted as a consequence of many other non-motor manifestations of PD, such as sleep, cognitive, and behavioral problems. Moreover, the investigation of circadian dysfunction in PD is hampered by the possible influence of many confounding factors, such as the motor fluctuations intrinsic to the disease and the influence of L-DOPA therapy. However, the presence of circadian imbalance in PD is well supported by circadian abnormalities described in many animal models of PD [for a review, see Ref. (79)].

In this complex scenario, a possible role in the pathogenesis of circadian problems described in PD patients can also involve

the eye, and in particular the mRGC system. At this regard, there is documentation of a strict interaction between the mRGCs and the dopaminergic amacrine cells (84), a depletion of dopamine levels, and a specific synuclein deposition, particularly in the inner retina (39, 51, 52). Furthermore, a possible direct link between the eye, through the regulation of the melatonin synthesis, and the motor symptoms of PD has been postulated by Willis (85), as supported by the amelioration of motor symptoms after light exposure in PD patients (86). A summary of the main circadian abnormalities in PD is reported in **Table 1**.

In this wide scenario, it is possible that many factors, including the influence of mRGCs on modulating circadian rhythms and sleep, may play a role in the pathogenesis of circadian and sleep problems in PD. A more detailed investigation of this system is warranted, especially in *de novo* PD cases to elucidating the mechanisms behind.

## BEYOND AD AND PD: HUNTINGTON'S DISEASE

Sleep and circadian dysfunction occur early in the disease course of Huntington's disease (HD) representing relevant non-motor symptoms of the disease [for a review, see Ref. (87)]. In particular, a delayed phase of rest–activity rhythm (88), an abnormal day–night ratio and melatonin rhythm (89), and consistent sleep fragmentation (90, 91) with increasing awakenings and reduced sleep efficiency have all been reported in HD.

Interestingly, the occurrence of sleep fragmentation and circadian misalignment in HD patients is relevant for aggravating the motor and cognitive problems of HD patients and bright light therapy improves motor and cognitive deficits in HD (90, 92). Moreover, even if there are evidences of neurodegenerative changes affecting the SCN in HD postmortem brain, the intact function of isolated SCN cells does not point to a primary central clock problem in the pathogenesis of circadian problems in HD, but more probably to a dysfunctional circuitry (87). Circadian abnormalities are also reported as early and prominent signs in the HD mouse models, the transgenic R6/2 and "knock-in" Q175 mice (93, 94). A summary of circadian abnormalities in HD is reported in **Table 1**.

Results on the possible occurrence of retinal degeneration in HD are contrasting, with some papers reporting the absence of retinal degeneration such as in the R6/2 mouse model (95) and others showing the presence of optic nerve degeneration (96, 97). In particular, a recent OCT study demonstrated the presence of temporal thinning in HD patients, which correlated with disease duration (97), with a pattern similar to PD and mitochondrial disorders (46, 47).

Interestingly, a recent study reported the occurrence of pupillary light response (PLR) dysfunction in R6/2 and Q175 mouse models, with a prevalent contribution of cone dysfunction in young–middle-aged mice and of mRGCs in old mice (98). In fact, a reduced PLR response is documented at low and moderate light intensity in young–middle-aged mice, whereas it is visible also at bright light in old mice, pointing to mRGC dysfunction. However, even if a significant reduction of melanopsin expression is evident in both mouse models also at early stages of the disease, the mRGCs are morphologically intact and do not show any signs of neurodegeneration. In particular, the aggregation of huntingtin protein is evident in a significant amount in the retina and in particular in the RGCs, but it is not recognized in the mRGCs except for the old animals, suggesting that mRGCs are relatively spared by neurodegeneration (98). These findings are in line with the findings in mitochondrial optic neuropathies, i.e., Leber's hereditary optic neuropathy and dominant optic atrophy (DOA), where we demonstrated a relative resistance of mRGCs to mitochondrial dysfunction (99) and relative sparing of the PLR (100). This similarity can be explained by the significant contribution of mitochondrial dysfunction in HD pathogenesis (101, 102), including the mitochondrial dynamics alterations seen in HD, in particular increased mitochondrial fission (103), similar to DOA due to OPA1 mutations where fusion is affected (104).

However, even if the mRGCs are more resistant to neurodegenerative changes occurring in HD, the evidence of retinal pathology and, in particular, of reduced melanopsin expression in the retina of these mice can be relevant to the pathogenesis of circadian dysfunction in HD. These findings in HD are further examples that link the eye to the brain in a continuous dialog.

## CONCLUDING REMARKS

In this review, we summarized the recent findings of optic nerve pathology and its possible link with circadian dysfunction in AD (4, 20, 105, 106), PD (5, 44), and HD (87, 96–98) focusing in particular on the possible role of mRGCs in the pathogenesis of circadian dysfunction in these neurodegenerative disorders.

We also underscore the differences in the patterns of optic nerve degeneration described in these disorders, which predominantly affect the magnocellular RGCs of the retina in AD (16, 21) and the parvocellular RGCs in PD (44, 46, 49) and HD (97), possibly explained by the predominant mitochondrial dysfunction documented in PD and HD. Similarities and differences are also discussed in regards to the circadian rhythm imbalance documented in AD and PD.

The presence of neuropathological hallmarks, i.e., β-amyloid plaques (30, 107, 108), α-synuclein (51, 52), and huntingtin (98) in the retina of these neurodegenerative disorders demonstrates that the retina is specifically affected by neurodegeneration and affords access to potential biomarkers of the disease.

## AUTHOR CONTRIBUTIONS

CLM was responsible for conception, design, drafting, and revision of the manuscript. FR-C, AS, and VC were responsible for conception and revision of the manuscript.

## FUNDING

This work was supported by the Ministry of Health Young Researcher project (GR-2013-02358026) (CLM), Research to Prevent Blindness (RPB), the International Foundation for Optic Nerve Diseases (IFOND), and NIH/NEI grant EY03040 (FR-C and AS). The authors also thank Dr. Gaetano Cantalupo for his help in figure editing.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 24 May 2017
doi: 10.3389/fneur.2017.00206

# The Retina in Multiple System Atrophy: Systematic Review and Meta-Analysis

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**Background:** Multiple system atrophy (MSA) is a rare, adult-onset, rapidly progressive fatal synucleinopathy that primarily affects oligodendroglial cells in the brain. Patients with MSA only rarely have visual complaints, but recent studies of the retina using optical coherence tomography (OCT) showed atrophy of the peripapillary retinal nerve fiber layer (RNFL) and to a lesser extent the macular ganglion cell layer (GCL) complex.

**Methods:** We performed a literature review and meta-analysis according to the preferred reporting items for systematic reviews and meta-analyses guidelines for studies published before January 2017, identified through PubMed and *Google Scholar* databases, which reported OCT-related outcomes in patients with MSA and controls. A random-effects model was constructed.

**Results:** The meta-analysis search strategy yielded 15 articles of which 7 met the inclusion criteria. The pooled difference in the average thickness of the RNFL was −5.48 μm (95% CI, −6.23 to −4.73; $p < 0.0001$), indicating significant thinning in patients with MSA. The pooled results showed significant thinning in all the specific RNFL quadrants, except in the temporal RNFL quadrant, where the thickness in MSA and controls was similar [pooled difference of 1.11 μm (95% CI, −4.03 to 6.26; $p = 0.67$)]. This pattern of retinal damage suggests that MSA patients have preferential loss of retinal ganglion cells projecting to the magnocellular pathway (M-cells), which are mainly located in the peripheral retina and are not essential for visual acuity. Visual acuity, on the other hand, relies mostly on macular ganglion cells projecting to the parvocellular pathway (P-cells) through the temporal portion of the RNFL, which are relatively spared in MSA patients.

**Conclusion:** The retinal damage in patients with MSA differs from that observed in patients with Parkinson disease (PD). Patients with MSA have more relative preservation of temporal sector of the RNFL and less severe atrophy of the macular GCL complex. We

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**Edited by:**
*Ivan Bodis-Wollner, SUNY Downstate Medical Center, United States*

**Reviewed by:**
*Shahnaz Miri, MedStar Georgetown University Hospital, United States*
*Patricija Van Oosten-Hawle, University of Leeds, United Kingdom*

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**Specialty section:**
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

**Received:** *14 February 2017*
**Accepted:** *27 April 2017*
**Published:** *24 May 2017*

**Citation:**
*Mendoza-Santiesteban CE, Gabilondo I, Palma JA, Norcliffe-Kaufmann L and Kaufmann H (2017) The Retina in Multiple System Atrophy: Systematic Review and Meta-Analysis. Front. Neurol. 8:206. doi: 10.3389/fneur.2017.00206*

**Abbreviations:** αSyn, α-synuclein; DLB, dementia with Lewy bodies; ERG, electroretinography; GCC, ganglion cell complex; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; MSA, multiple system atrophy; MSA-C, multiple system atrophy-cerebellar; MSA-P, multiple system atrophy-parkinsonian; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; PD, Parkinson disease; PSP, progressive supranuclear palsy; RGC, retinal ganglion cells; RNFL, peripapillary retinal nerve fiber layer; UMSARS, united multiple system atrophy rating scale; VEP, visual evoked potentials abnormalities.

hypothesize that in patients with MSA there is predominant damage of large myelinated optic nerve axons like those originating from the M-cells. These large axons may require higher support from oligodendrocytes. Conversely, in patients with PD, P-cells might be more affected.

**Keywords: multiple system atrophy, retina, alpha-synuclein, optical coherence tomography, ganglion cell layer, retinal nerve fiber layer**

## INTRODUCTION

Multiple system atrophy (MSA) is a rare, adult-onset fatal synucleinopathy, a group of neurodegenerative disorders driven by abnormal intracellular aggregation of misfolded hyperphosphorylated fibrillar α-synuclein (αSyn) (1). In MSA, the initial abnormal αSyn deposition occurs in oligodendroglial cells forming glial cytoplasmic inclusions while in other synucleinopathies αSyn deposits occur in neurons forming Lewy bodies and Lewy neurites (2). MSA has common motor and non-motor clinical features with Parkinson disease (PD), but the clinical course of MSA is usually rapid with mean survival below 10 years from diagnosis and with no effective symptomatic or neuroprotective treatments (3–5).

Visual symptoms are not frequent in patients with MSA, but recent studies using optical coherence tomography (OCT) showed progressive retinal thinning with a distinctive pattern and anatomic distribution, which has now been confirmed in postmortem studies (6). Because current candidate biomarkers for MSA from blood, cerebrospinal fluid, and brain or cardiac imaging are neither sensitive nor specific or insufficiently explored (7), OCT-detected retinal abnormalities could emerge as a useful biomarker of disease progression (8). In this article, we briefly review the normal anatomy of the retina and perform a literature review of retinal abnormalities as a biomarker in patients with MSA and a meta-analysis on the main results of OCT studies in patients with MSA. Finally, we discuss putative pathological mechanisms that may explain the observed retinal abnormalities in these patients.

### Anatomy of the Retina

The retina derives embryologically from the neural tube and is part of the central nervous system. Because it is attached to the posterior surface of the ocular globe, the retina can be easily explored through the transparent media of the eye. The cellular architecture of the retina highly resembles the cerebral cortex with three layers of cells (instead of six) connected vertically by photoreceptors, bipolar cells, and ganglion cells, and horizontally by modulating interneurons. The interneurons group includes horizontal cells modulating the conduction between photoreceptors – rods and cones – and bipolar cells in the outer plexiform layer (OPL), and amacrine cells, modulating the conduction between bipolar cells and ganglion cells in the inner plexiform layer (IPL). Amacrine, bipolar, and horizontal cells are located in the inner nuclear layer (INL). The combination of the macular IPL, the macular ganglion cell layer (GCL), and the thin nerve fiber layer at the macula is referred to as ganglion cell complex (GCC) (**Figure 1**). Some OCT devices (e.g., Zeiss Cirrus®), however, do not include the retinal nerve fiber layer at the macula when assessing the GCC.

The visual information is highly processed and segregated in the retina and finally conducted using sub-populations of retinal ganglion cells (RGC), whose axons converge in the optic nerve. The classification of RGC sub-populations has evolved from the seminal morphological criteria of Ramón y Cajal (9) to more sophisticated criteria based on morphological, molecular, and genetic properties of the cells, particularly in murine models, with at least 25 RGC sub-populations identified so far (10). Based on their projections and functions, the classification of RGC can be simplified into four main sub-populations:

(a) Midget RGC (80%), projecting to the parvocellular layers of the lateral geniculate body (parvocellular pathway; P-cells). In this review, we refer to these RGC as P-cells, based on their anatomical projections in the lateral geniculate body.
(b) Parasol RGC (10%), projecting to the magnocellular layers of lateral geniculate body (magnocellular pathway; M-cells). In this review, we refer to these RGC as M-cells, based on their anatomical projections in the lateral geniculate body.
(c) Bistratified ganglion cells (10%), projecting to the koniocellular layers of lateral geniculate body (K pathway).
(d) Intrinsically photosensitive ganglion cells, projecting to suprachiasmatic nucleus.

M-cells, with wide retinal dendritic fields predominantly present in peripheral retinal regions, are specialized in motion detection and low spatial frequency achromatic contrast sensitivity. P-cells, with smaller dendritic fields and concentrated in the central retina (macula), are responsible for visual acuity, color discrimination, and high spatial frequency chromatic/achromatic contrast sensitivity (11). Axons from P-cells enter the temporal portion of the optic nerve in thinner nerve bundles, whereas axons from M-cells enter the inferior, superior, and nasal portions of the optic nerve in thicker bundles. Oligodendroglial cells myelinate the axons of M-cells and P-cells only after they exit the eye when crossing the lamina cribrosa (12).

### The Retina As a Biomarker in Neurodegenerative Disorders

Structural and functional changes of the retina are increasingly recognized as potential biomarkers for early diagnosis, prognosis, and progression of neurodegenerative disorders (13–15). The structure of the retina can be easily and non-invasively assessed with OCT, whereas retinal function can be evaluated with electrophysiological techniques, including visual evoked potentials (VEP) and electroretinography (ERG), and psychophysical

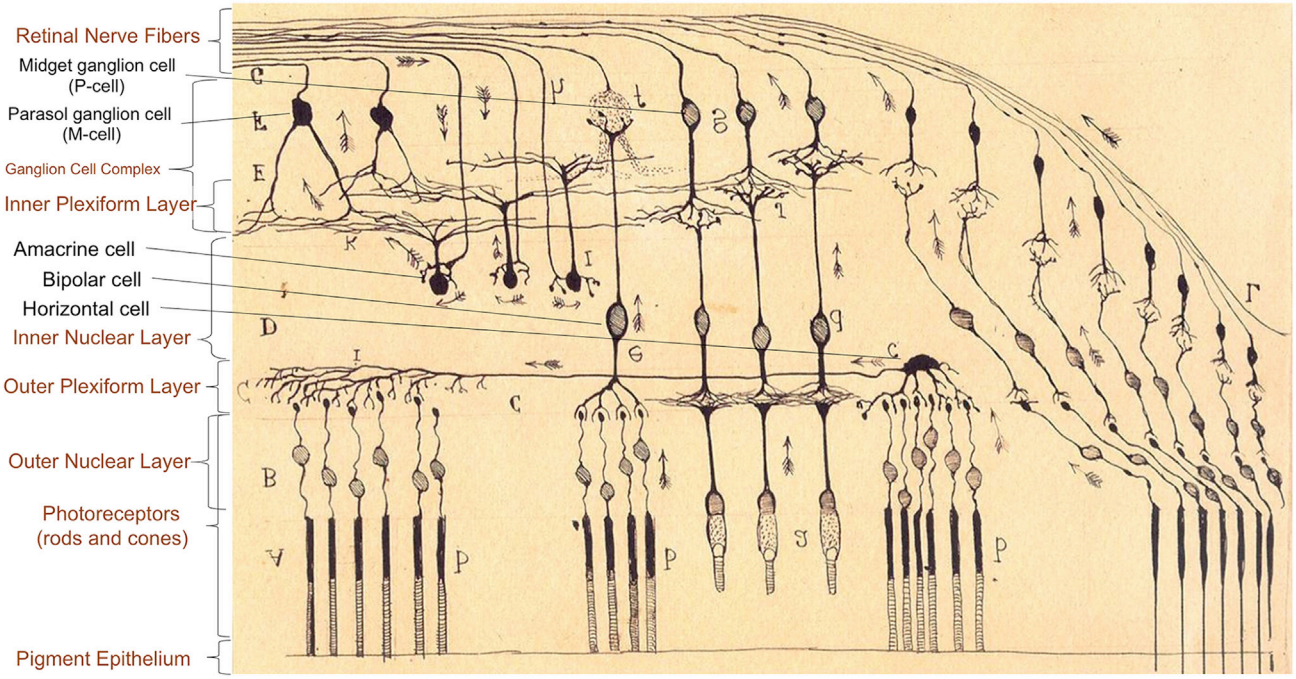


**FIGURE 1 | Anatomy of retinal layers as described by Ramón y Cajal (9), Spanish neuroscientist pioneer in the investigation of the microscopy structure of the nervous system, including the retina, and Nobel Prize Awardee (1906)**. There are three layers of cells connected vertically (photoreceptors, bipolar cells, and ganglion cells) and horizontal interneurons modulating the signal conduction at two levels: horizontal cells in the conduction between photoreceptors (rods and cones) and bipolar cells in the outer plexiform layer, and amacrine cells between bipolar cells and ganglion cells in the inner plexiform layer. Large ganglion cells [retinal ganglion cells (RGC) projecting to the magnocellular pathway, M-cells] are specialized in motion detection and low spatial frequency achromatic contrast sensitivity. Smaller RGC with thinner axons (RGC projecting to the parvocellular pathway, P-cells) are concentrated in the central retina (macula), and they are responsible for visual acuity, color discrimination, and high spatial frequency chromatic/achromatic contrast sensitivity.

methods, such as high and low contrast visual acuity, color, and movement perception. Pathological studies showed retinal damage in patients with Alzheimer disease (16), multiple sclerosis (17), idiopathic PD (18, 19), MSA (6), and mitochondrial neurodegenerative disorders (20).

In idiopathic PD and dementia with Lewy bodies (DLB), the most common synucleinopathies, visual symptoms are relatively frequent with ~80% of patients with PD reporting some visual-related problem such as double vision, difficulty to read despite adequate ocular refraction, gait freezing in narrow spaces, abnormal judgment of objects while walking, or visual hallucinations (21–24).

*In vivo* studies using OCT in non-human primate models of PD (MPTP-treated) (25) as well as in patients with PD have shown specific neurodegeneration of internal retinal layers. Initial reports showed atrophy of the peripapillary retinal nerve fiber layer (RNFL), especially in its temporal and temporal–inferior sectors (26, 27). Subsequent studies highlighted the presence of a specific profile of atrophy of inner retinal layers in the macula (28, 29). Recent publications have described flattening of the foveal pit affecting the foveal avascular zone of the macula (30, 31), a region with higher density of amacrine cells (32). Retinal damage in PD, as measured by OCT, has been associated with poorer visual function (33, 34), abnormal VEP and ERG (35), longer disease duration and higher symptomatic burden including dementia (36, 37), and visual hallucinations (38).

In line with OCT findings, histopathological evidence in animal models of PD showed a reduction in the number of dopaminergic cells (39–41). Postmortem studies of the retinas from patients with PD showed thinning of inner retinal layers, especially in the INLs, with intracellular and extracellular aggregates of αSyn in the GCL, IPL, and INL, not present in controls, suggestive of Lewy bodies (18). Another group showed αSyn deposition within retinal fibers penetrating the inner part of the retina, involving the RNFL, the GCL, and the IPL (19). This is in contrast to patients with MSA, in whom no retinal deposits of αSyn have been identified in spite of severely reduced density of RGC (6).

## Visual Abnormalities in MSA

In clinical practice, patients with MSA do not typically complain about specific visual problems, in contrast to the relatively frequent visual symptoms in patients with PD and DLB. When patients with MSA report eye-related symptoms, these are due to efferent (motor) visual system abnormalities, such as blepharospasm, blurry vision, or diplopia as a consequence of oculomotor abnormalities (e.g., excessive square jerks, mild vertical supranuclear gaze palsy, nystagmus, saccadic hypometria, impaired smooth pursuit, or visual oculocefalic reflex suppression) (42–46). The current consensus criteria on the diagnosis of MSA consider the presence of visual hallucinations not induced by drugs, a red flag against the diagnosis of MSA (47).

**TABLE 1 | Summary of retinal OCT studies in MSA.**

| Reference | OCT device | Number of subjects | RNFL global (μm) | RNFL temporal (μm) | RNFL inferior (μm) | RNFL superior (μm) | RNFL nasal (μm) | TMT global (μm) | GCC global (μm) |
|---|---|---|---|---|---|---|---|---|---|
| Ahn et al. (50) | Spectralis® (RNFL)/ OPKO OTI® (macula) | 15 MSA<br>27 controls | 94.39 (13.92) | 74.61 (12.71) | 119.46 (21.44)* | 114.75 (23.83) | 68.79 (13.34) | 268.78 (22.33) | – |
| | | 23 MSA<br>44 controls | 102.15 (10.02) | 79.32 (12.55) | 132.26 (18.24) | 124.58 (14.65) | 72.43 (10.08) | 273.74 (18.33) | – |
| Mendoza-Santiesteban et al. (51) | Cirrus® | 24 MSA<br>35 controls | 84.6 (5.0)*<br>89.8 (6.5) | 59.7 (9.5)<br>62.0 (7.4) | 108.1 (9.8)*<br>117.9 (10.7) | 105.3 (11.6)<br>109.4 (10.2) | 67.8 (5.9)<br>70.7 (8.8) | –<br>– | 76.0 (6.3)*<br>80.6 (5.2) |
| Schneider et al. (56) | Cirrus® | 12 MSA<br>41 controls | –<br>– | –<br>– | –<br>– | –<br>– | –<br>– | 267.5 (9.4)<br>277.5 (15.3) | 66.7 (7.4)<br>72.4 (6.7) |
| Fischer et al. (54) | Spectralis® | 12 MSA<br>10 controls | 93.18 (8.16)*<br>97.20 (2.66) | 79.47 (16.17)<br>75.30 (5.08) | 98.33 (14.81)<br>123.7 (3.12) | 114.06 (16.1)<br>117.8 (2.82) | 61.76 (13.46)*<br>71.10 (1.52) | 228.82 (24.86)<br>232.30 (10.24) | –<br>– |
| Albrecht et al. (52) | Spectralis® | 19 MSA<br>35 controls | 93.79 (1.92)<br>99.13 (1.59) | 72.37 (3.45)<br>73.89 (2.02) | 118.4 (4.16)<br>126.7 (3.06) | 115.9 (3.7)<br>121.6 (2.78) | 68.18 (2.23)<br>71.99 (2.34) | 308.2 (4.13)<br>317.6 (2.69) | 96.08 (1.95)<br>98.7 (1.60) |
| Pula et al. (55) | Spectralis® | 5 MSA | 100 (11) | – | – | – | – | 3 mm/6 mm<br>314 (12)*/285 (15) | – |
| | | 27 controls | 98 (9) | – | – | – | – | 339 (17)/295 (17) | – |
| Fischer et al. (53) | Spectralis® | 10 MSA<br>10 controls | 91.30 (1.45)*<br>97.2 (1.45) | 83.40 (3.25)<br>75.30 (3.25) | 114.15 (3.43)<br>123.7 (3.43) | 111.05 (3.04)<br>117.8 (3.04) | 61.70 (1.63)<br>71.10 (1.63) | 234.20 (5.14)<br>232.30 (5.14) | –<br>– |

*$p < 0.05$ MSA versus controls.

*OCT, optical coherence tomography; MSA, multiple system atrophy; RNFL, peripapillary retinal nerve fiber layer thickness; TMT, total macular thickness; GCC, ganglion cell complex (ganglion cell layer + inner plexiform layer) thickness; global, average of all measurements; temporal, inferior, superior and nasal, sectors of RNFL.*

To have a statistical synthesis of the results of all retinal OCT studies in patients with MSA published to date, we performed a meta-analysis summarizing the differences reported in overall and sectorial RNFL thickness in MSA compared to healthy controls. Macular OCT measures were not included in the meta-analysis since acquisition protocols were highly variable across studies (**Table 1**). In contrast, acquisition and measurement protocols for RNFL thickness were consistent between the two most extensively used OCT apparatus (Heidelberg Spectralis® and Zeiss Cirrus®) used in MSA studies. In fact, the agreement between those two specific devices to differentiate normal or abnormal RNFL thickness has been already demonstrated in ophthalmological diseases such as glaucoma (48).

## METHODS

The meta-analysis was prepared according to the preferred reporting items for systematic reviews and meta-analyses guidelines (49). Articles on OCT and MSA were identified by searches of PubMed through January 1, 2017. We included only articles in English. The following search terms were used: "multiple system atrophy," "MSA," "Shy-Drager," "striatonigral degeneration," "olivopontocerebellar," "autonomic failure," "optical coherence tomography," "OCT," and "retina." We also reviewed the reference lists of the retrieved articles. We did not include unpublished data or data from abstracts.

For the meta-analysis, articles were evaluated independently by two reviewers (Iñigo Gabilondo and Jose-Alberto Palma) who extracted the following data from each study: first author, year of publication, OCT device type, study participants (MSA and controls), and OCT results (mean and standard deviation) on the thickness (in μm) of the following retinal areas: average RNFL, temporal RNFL, inferior RNFL, superior RNFL, and nasal RNFL. Case reports were excluded.

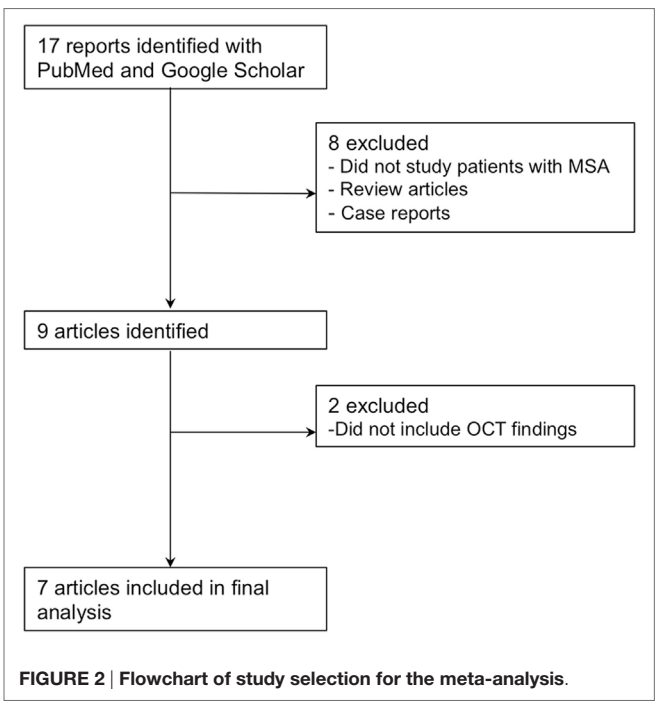


**FIGURE 2 | Flowchart of study selection for the meta-analysis.**

### Statistical Analysis

We used combined mean difference as a common measure of association between MSA and retinal thickness. The pooled

difference in the thickness of specific retinal areas between MSA and controls and 95% confidence intervals were obtained by using a random-effects model. We used a random-effects model rather than a fixed-effects model because of the high likelihood of heterogeneity between study variance. The heterogeneity of effect size estimates across studies was described with the $I^2$ index

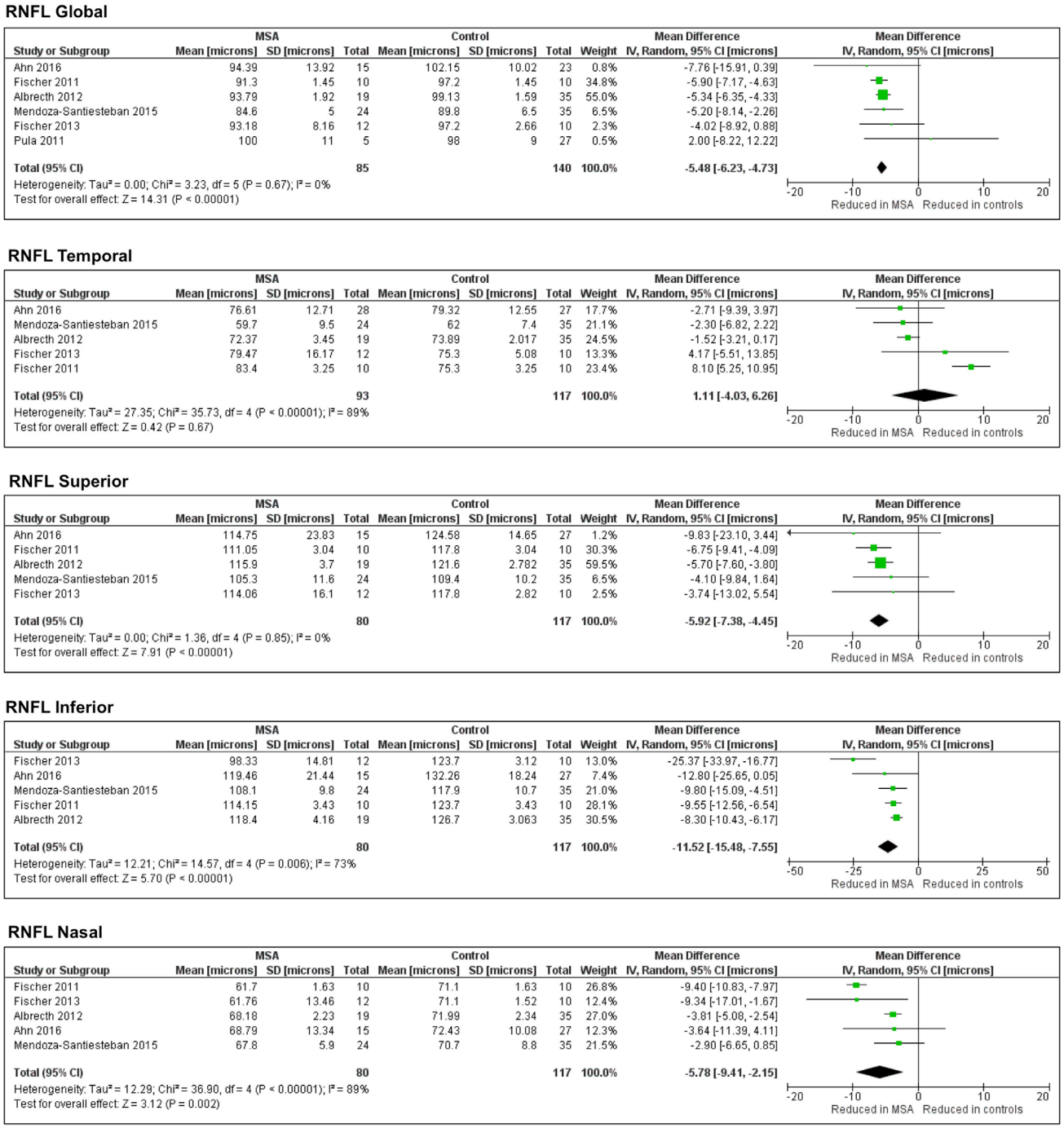


**FIGURE 3 | Forest plots showing the pooled difference in the average thickness of the retinal nerve fiber layer in global and specific quadrants of multiple system atrophy (MSA) versus controls**.

(with values of 25, 50, and 75% considered low, moderate, and high, respectively). Sensitivity analysis was performed using the leave-one-out approach. Analyses were performed with Review Manager 5.3 (Cochrane Collaboration, Nordic Cochrane Center, Denmark). $p < 0.05$ was considered as statistically significant, indicating significant thinning in patients with MSA versus controls.

# RESULTS

## Meta-Analysis Results

As shown in **Figure 2**, the primary search strategy yielded 15 articles of which 7 met the inclusion criteria. **Table 1** shows the characteristics of the seven identified studies.

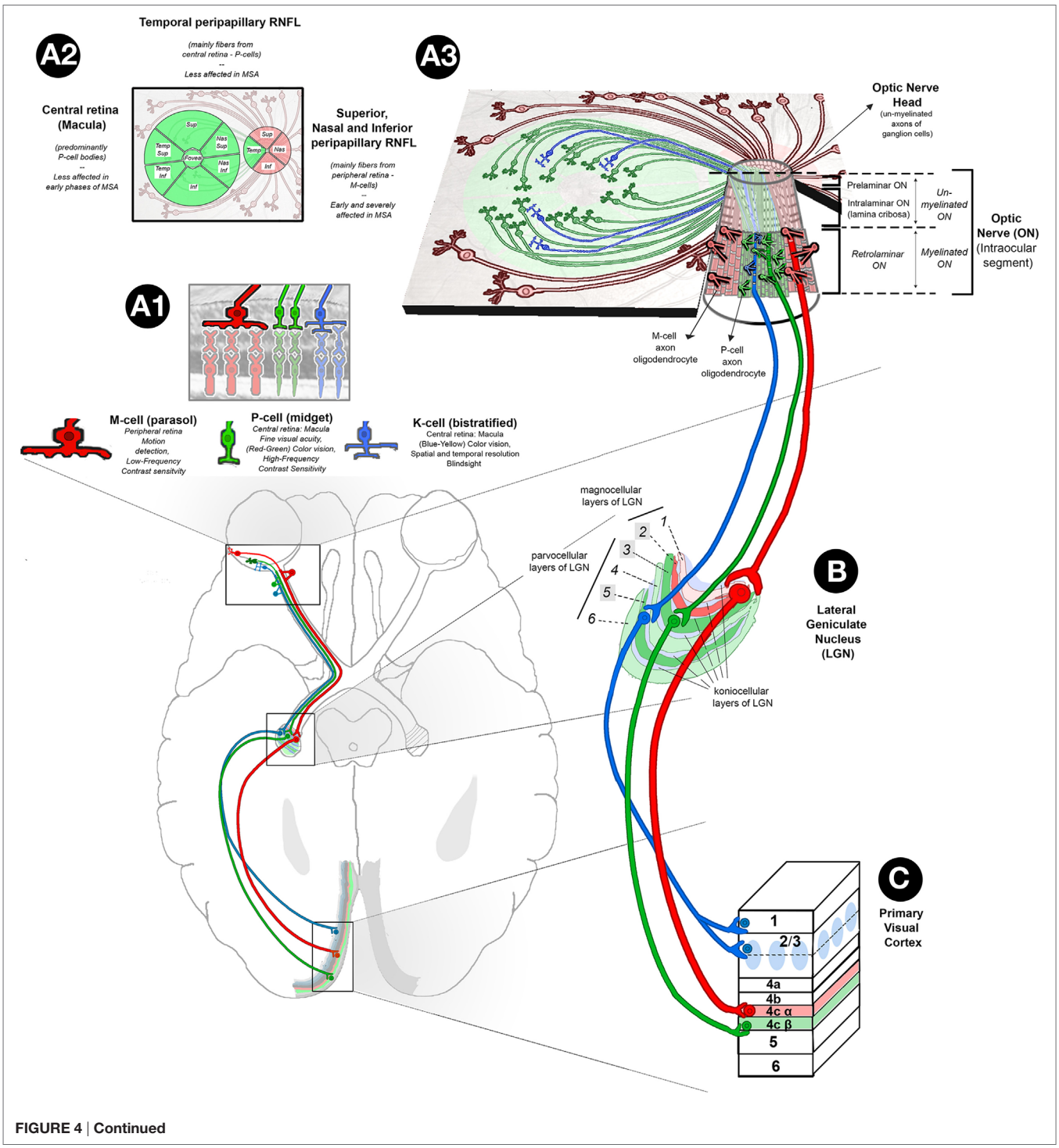


**FIGURE 4** | **Continued**

**FIGURE 4 | Continued**

**Retinal abnormalities in MSA**. **(A1)** Based on their morphology, functions, and projections to specific layers of LGN, there are three main types of ganglion cells in the retina: (1) parasol cells or M-cells (represented in red) (10%): their cell bodies are predominantly located in the peripheral retina, and their axons project through RNFL to sup, nas, and info sectors of the ON head, ultimately reaching the magnocellular layers of the LGN; M-cells are responsible for movement discrimination and low-frequency contrast sensitivity; (2) midget ganglion cells, or P-cells (represented in green) (80%): their cell bodies are predominantly located in the central retina (macula), and their axons project through the papillomacular bundle of the RNFL to the temp sector of the ON head, reaching parvcellular layers of the LGN; their function has been related to fine visual acuity (red–green) color vision and high-frequency contrast sensitivity; and (3) bistratified cells, or K-cells (represented in blue) (10%): their distribution in the retina is similar to P-cells, and their axons synapse with koniocellular layers of the LGN; their function is related to blue–yellow color vision, different aspects of spatial and temp resolution and blind sight. **(A2)** Retinal map of the distribution of P-cell (green color) and M-cell (red color) bodies and axons. **(A3)** Tridimensional representation of retina P-, M-, and K-cell fibers and their organization in the intraocular ON (info center): the intraocular ON is the first segment of the ON after ON head, in which RNFL axons penetrate the neural retina, choroid, and sclera to form the extraocular ON. The intraocular ON is divided from proximal to distal in preliminar, intralaminar, and a retrolaminar portions. The intralaminar portion contains the lamina cribrosa, a multilayered network of collagen fibers that insert into the scleral canal wall. When un-myelinated axons of P, K, and M-ganglion cells reach the lamina cribrosa, they become myelinated by the myelin sheath of ON oligodendrocytes, each of them covering several ganglion cell axons. According to current evidences on optical coherence tomography, in MSA, sup, nas, and info sectors of peripapillary RNFL are affected early and severely while temp sectors of peripapillary RNFL and central macular ganglion cell layer are relatively spared. This finding suggests a specific pattern of retina damage in MSA in which M-cells are specifically affected. This hypothesis is physiopathologically plausible, since MSA is a primary oligodendropathy and M-cells with their bigger axons may require higher myelination support from oligodendrocytes. **(B)** The LGN has layers of magnocellular cells and parvocellular cells that are interleaved with layers of koniocellular cells. In humans, the LGN is normally described as having six distinctive layers. The inner two layers (1 and 2) are magnocellular layers, while the outer four layers (3, 4, 5, and 6) are parvocellular layers. Koniocellular cells are located in additional set of layers found ventral to each of magnocellular and parvocellular layers. Layers 2, 3, and 5 receive inputs from ganglion cells of ipsilateral retina (highlighted in brighter colors), and layers 1, 4, and 6 receive ganglion cell axons from contralateral retina that crossed the chiasm. **(C)** Primary Visual Cortex (V1). P-cells project to parvocellular layers of the LGN and on to layer 4Cβ of V1. M-cells project to magnocellular layers of the LGN and on to layer 4Cα of V1. K-cells project to koniocellular layers of the LGN and on to the cytochrome oxidase-expressing patches (or blobs) of layer 2/3 and to layer 1. Abbreviations: sup, superior; nas, nasal; temp, temporal; info, inferior; MSA, multiple system atrophy; LGN, lateral geniculate nucleus; RNFL, retinal nerve fiber layer; ON, optic nerve.

### Retinal Nerve Fiber Layer in MSA

The pooled difference in the average RNFL thickness between controls and MSA was −5.48 μm (95% CI, −6.23 to −4.73; $p < 0.0001$), indicating a significant thinning in controls versus MSA. The pooled results showed significant thinning in MSA versus controls in all the specific RNFL quadrants, except in the temporal RNFL quadrant, where the thickness between MSA and controls was not different [pooled difference between controls and MSA was 1.11 μm (95% CI, −4.03 to 6.26; $p = 0.67$)] (**Figure 3**).

### Sensitivity Analysis and Publication Bias

Of the seven studies included in this meta-analysis, two (50, 51) used Cirrus®. The remaining five (52–56) used Spectralis®. To clarify the potential effect on the pooled results caused by the different devices, we conducted a sensitivity analysis to explore potential sources of heterogeneity. After excluding the two studies that used Cirrus®, all the results remained unchanged. Because of the small number of study retrieved, no publication bias analysis was performed.

## Literature Review Results

### Electrophysiology in MSA

The initial evidence of the dysfunction of visual pathways in MSA was obtained with visual electrophysiology studies (57, 58). These found significant inter-eye difference in contrast sensitivity and latency delay in PD, which were not present in patients with MSA. One of these studies specifically evaluated retinal integrity in six patients with MSA, 12 patients with PD, and 33 healthy controls using flash and pattern ERG (in addition to VEP and psychophysical contrast thresholds, contrast discriminations and reaction times) (59). This study disclosed ERG abnormalities in patients with MSA, although much less severe than in patients with PD. Another study including six patients with MSA and 12 with PD showed that the chromatic pattern-reversal ERG is spared in MSA, in contrast to PD (60).

### OCT in MSA

The first OCT study in patients with MSA (53) included 10 patients with MSA and 10 age-matched controls. The average peripapillary RNFL (RNFL) (in circular B-scans centered in optic disk) and the total retinal thickness (in two linear B-scans at the foveola) were studied. The investigators found that the global RNFL thickness was significantly reduced in MSA patients compared to controls, particularly in the nasal quadrant. No differences in total retinal thickness were found.

Another study published shortly after (55) included five patients with MSA-C and 27 healthy controls. The study measured average RNFL thicknesses and global and sectorial total macular thickness, finding that global macular thickness was significantly reduced in MSA-C versus controls only in the 3-mm (but not in the 6-mm) diameter ring as well as in the temporal sector of the 6-mm macular ring. Unfortunately, no information on specific sectors of the RNFL was included in this study.

In 2012, Albrecht and colleagues (52) performed OCT studies in 19 patients with MSA, 40 patients with PD, 10 with corticobasal degeneration, 15 with progressive supranuclear palsy (PSP), and 35 controls. The results showed significant atrophy of the peripheral macula in patients with MSA compared to controls, but no differences in total or central macular or RNFL thickness. This study evaluated for the first time differences in thickness of deeper retinal layers of the macula using a semi-automatically segmented single B-scan situated in the middle of the fovea. These included the GCL and IPL complex (GCC + IPL, also known as the GCC), the INL, the OPL and the outer nuclear layer (ONL). No differences between MSA and controls were found in any of these layers.

Another study in 2013 evaluated 12 patients with MSA and 10 age-matched healthy controls (54). The study did not find differences in foveal thickness or global RNFL. However, the nasal RNFL was significantly thinner in MSA. In addition, the authors found no association between any OCT measurements, and visual field abnormalities, disease severity as measured by the United Multiple System Atrophy Rating Scale (UMSARS), and disease duration. It is unclear if, in this study, the authors included data of patients from their 2011 study (53).

In 2014, Schneider and colleagues (56) evaluated retinal damage in the macula and its layers using OCT in MSA ($n = 12$), PSP ($n = 16$) and PD ($n = 65$) patients, and 41 controls. They found no differences in the RNFL, GCC, and the INL in patients with MSA versus controls. Interestingly, they did find a significant thickening in the ONL and OPL of MSA versus controls, whereas in patients with PSP the ONL was thinner and the OPL was ticker than controls. The authors suggested that the ONL/OPL ratio could be useful to distinguish MSA from PSP, with high sensitivity (88%) and specificity (91%). This same study found no association between retinal thickness and neurological disability scores in MSA.

In 2015, we (51) published a cross-sectional study including 24 MSA, 20 PD patients, and 35 healthy controls. We found no differences in best-corrected high-contrast visual acuity and color vision between MSA and controls. OCT showed thinner RNFL (average and inferior quadrant) and thinner GCC in the macular cube in MSA patients. We also observed a tendency toward thinner GCC globally and thinner RNFL for all quadrants, especially for temporal RNFL in patients with PD compared to MSA. This preferential atrophy of the temporal RNFL quadrant in PD was also found when compared to controls.

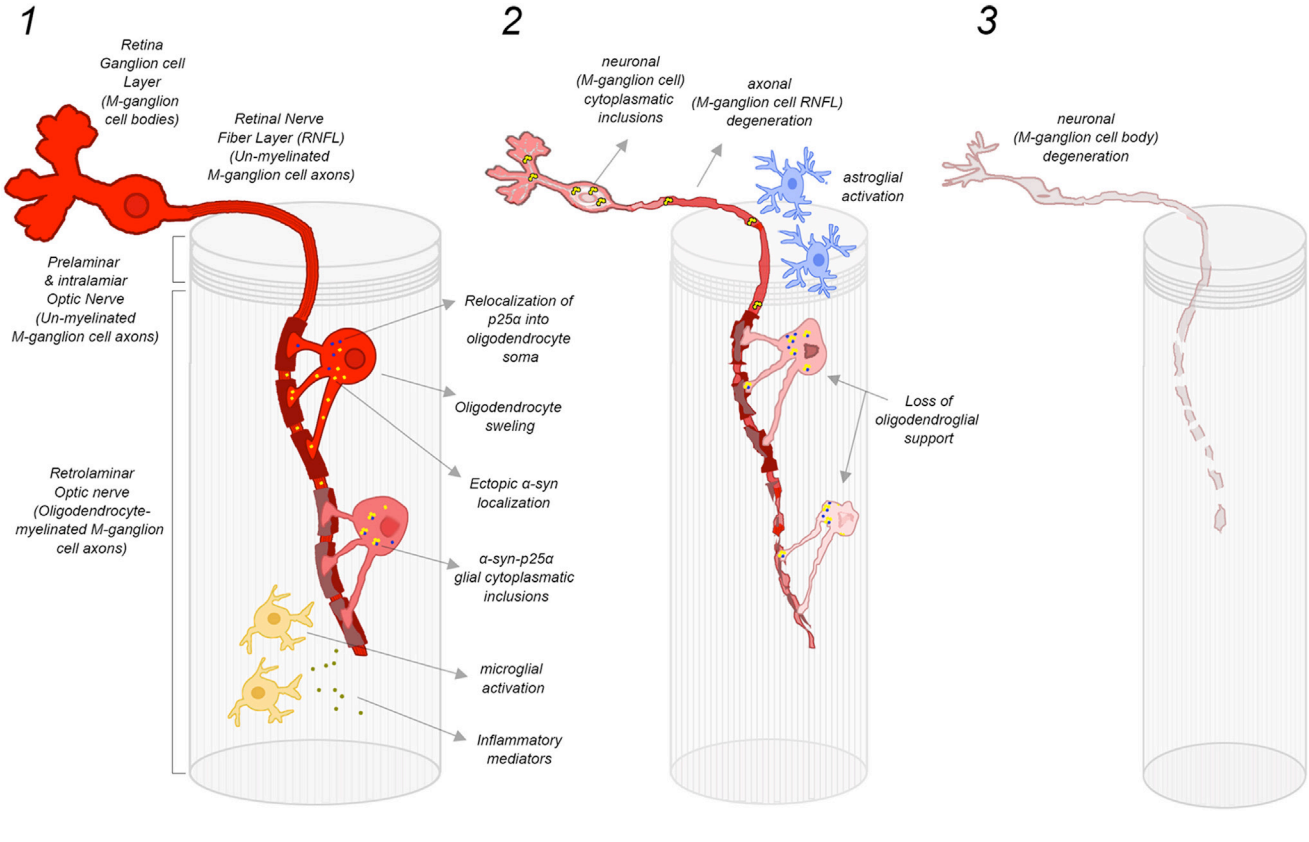


**FIGURE 5** | **Damage mechanisms and progression of retinal M-ganglion cell degeneration in MSA**. (1) Early stage of MSA: oligodendrocytes located in the preliminary ON provide myelin sheath to several P-cell and M-cell axons. In the case of M-cells, the level of myelination support is particularly prominent since their axons are larger. ON oligodendrocytes in MSA are potentially susceptible to follow the same cellular pathological cascade that has been proposed for brain oligodendrocytes, with an initial relocalization of p25α into oligodendrocyte soma and ectopic localization of αSyn, leading to a progressive oligodendrocyte swelling. The formation of α-syn-p25α glial inclusions may induce the activation of microglia and the release of inflammatory factors, which further contributes to oligodendrocyte and myelin degeneration. This early oligodendrocyte damage may induce also early injury of M-cell axons favoring the early atrophy of superior, nasal, and inferior sectors of ON head RNFL; (2) Advanced stage of MSA: the severe degeneration of oligodendroglia leads to a loss of oligodendroglial support, which promotes the degeneration of axons of M-ganglion cells. In addition, there is a liberation of misfolded αSyn by oligodendrocytes to extracellular space, which may be taken by adjacent neurons to form misfolded αSyn inclusions within axons and cell bodies of M-cells. Misfolded αSyn inclusions within M-cells further promote neuronal dysfunction and neurodegeneration, with a reactive activation of local ON astroglia. In this phase, there is a severe damage of M-cell axons in the retina (superior, nasal, and inferior sectors of ON) that may also involve to a lesser extent P-cell axons (atrophy of temporal sector of ON RNFL) and their cell bodies (macular GCL atrophy); and (3) End-stage MSA: there is a severe degeneration of M-cell axons and cell bodies that extend also to P-cell axons and bodies, reflected in the retina as a widespread atrophy of peripapillary RNFL (still more prominent in superior, nasal, and inferior sectors) and macular GCL. Abbreviations: MSA, multiple system atrophy; ON, optic nerve; αSyn, α-synuclein; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer. Figure inspired by Ref. (1).

Ours (51) was the first study to document progressive longitudinal changes in the retina in patients with MSA, as 13 of the initial 24 MSA patients were also followed up overtime with two to seven visits for a mean of 12 months (maximum follow-up of 26 months). We observed a rate of thinning in the RNFL of −3.72 μm (−4.32%) per year. This RNFL reduction rate was higher than the one of healthy subjects (−0.33 μm/year) and also notably higher than the one reported in multiple sclerosis (−2.0 μm/year). The macular GCC also thinned, although to a much lesser rate of −1.8 μm (−2.52%) per year. Longer follow-up periods were associated with more intense thinning of the RNFL and GCC. In an attempt to open the door to using OCT in clinical trials of MSA, we also estimated the required number of patients for clinical trials in order to use the RNFL thickness as an objective outcome measure.

The most recent study using OCT (50) analyzed the RNFL in 15 patients with MSA and 27 controls, and total macular thickness in 23 MSA and 44 controls. This study showed a significant thinning of the RNFL and total retinal thickness in outer superior macular sectors in patients with MSA. Total macular thickness in patients with MSA was associated with their UMSARS and Global Disability Score. For unclear reasons, the authors used different OCT devices for the acquisition of the RNFL thickness (Spectralis®) and the macular thickness (OPKO OTI®).

The reviewed studies of OCT in MSA have some common limitations:

(a) The highest sample size so far has been 24 MSA patients (51), which is relatively low, although MSA is a rare disease.
(b) Only one study (51) measured longitudinal changes over time; all other studies were cross-sectional with no follow-up.
(c) The use of different OCT devices (Cirrus®, Spectralis®, OPKO OTI®) and algorithms may lead to heterogeneous results. In this regard, the acquisition protocol of macular measurements (e.g., image resolution, number and dimension of slices, analyzed areas from those slices, layers analyzed, and segmentation methods) varied considerably among studies, and in some studies certain macular measurements were not reported.
(d) The statistical analysis of the OCT results was markedly different: most studies averaged the results of both eyes (51, 54–56); two considered the results of each eye as independent values (50, 52), whereas one study used only the results of the right eye (53).

## DISCUSSION

Although afferent visual symptoms are uncommon in patients with MSA, OCT and electrophysiological studies support the presence of retinal abnormalities in these patients.

The retinal damage in patients with MSA appears to follow a different pattern to that observed in those with PD. While in PD patients the atrophy of temporal RNFL sectors and internal retinal layers (i.e., GCC) at the parafoveal region are prominent, in patients with MSA the inferior, superior and nasal RNFL sectors are more affected than in PD.

The dissimilarities between PD and MSA patients at the clinical and retinal level could be explained by differences in the preferential damage of P-cells versus M-cells (51). P-cells predominate in the central macular region (where the macular GCC is measured), their axons project to the temporal portion of the retinal nerve fiber layer and they are highly related to color discrimination, visual acuity, central visual field sensitivity, and contrast sensitivity for high spatial frequencies. On the other side, axons from M-cells (with cell bodies situated in peripheral macula and retina) are located in the superior, nasal, and inferior regions around the optic nerve (where the RNFL is measured). These M-cells relay information about achromatic vision, peripheral visual field sensitivity, motion detection, and contrast sensitivity for low spatial frequencies. The fact that MSA patients typically have normal visual acuity and color vision in combination with inferior RNFL atrophy may indicate that, in

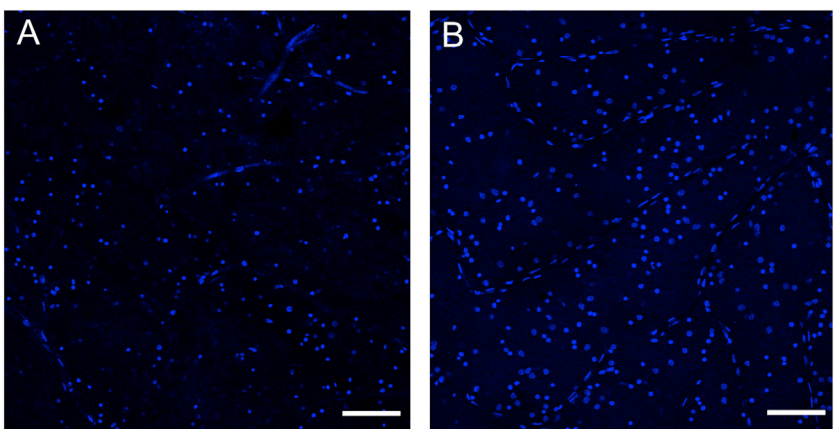


**FIGURE 6 | Loss of retinal ganglion cells in the peripheral retina of a patient with multiple system atrophy (MSA)**. Confocal images of representative areas of whole-mounted retinae (superior–temporal area of the far peripheral retina; distance from the ora serrata: 1–5 mm) labeled with the blue fluorescent Hoechst marker of a patient with MSA **(A)** and an age-matched normal subject **(B)**. Scale bar is 100 μm. The number of ganglion cells is markedly reduced in MSA compared to the control. See Ref. (6) for additional information.

MSA, M-cells are more affected than P-cells. This is in contrast to PD patients, who have preferential atrophy of temporal RNFL sectors and higher central macular GCC atrophy (compared to MSA), and is in keeping with their relatively frequent complains of visual problems, such as decreased visual acuity, impaired color discrimination, defective motion perception, and visual hallucinations (**Figure 4**). The hypothesis of a preferential injury of P-cells in PD and M-cells in MSA is still unproven, but it may be related to a predominant damage of optic nerve axons in MSA that being especially coarse and myelinated (like those from M-cells) require high support from oligodendrocytes (**Figure 5**).

## CONCLUSION

Multiple system atrophy is a rare, adult-onset fatal synucleinopathy driven by a primary dysfunction of CNS oligodendrocytes. While efferent visual or oculomotor symptoms are relatively common in MSA patients, most MSA patients rarely report afferent visual symptoms. Despite the paucity of symptoms, our meta-analysis shows that patients with MSA have significantly decreased RNFL in all, except in the temporal quadrant. Two publications also showed that these retinal thinning worsens with disease progression and severity (50, 51). Pathological confirmation of reduced peripheral RGC in patients with MSA has been recently reported (6) (**Figure 6**).

The retinal damage in MSA patients follows a different pattern to that observed in those with PD. Patients with MSA have more prominent atrophy of inferior sectors of the RNFL and more preserved central macular GCC. Patients with PD have more atrophy in the temporal RNFL and the GCC layers of the central–parafoveal macula. We hypothesize that in patients with MSA there is predominant damage of large myelinated optic nerve axons like those from the M-cells. These large axons may require higher support from oligodendrocytes. In contrast, in patients with PD, P-cells appear to be more affected. Further studies with pathological confirmation are required to validate these findings.

## AUTHOR CONTRIBUTIONS

CM-S, IG, and J-AP: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision. LN-K and HK: acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content.

## FUNDING

The study was funded by NIH (U54 NS065736), Michael J. Fox Foundation, and Dysautonomia Foundation.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 05 July 2017
doi: 10.3389/fneur.2017.00324

# Neurochemical Systems of the Retina Involved in the Control of Movement

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Recent studies have revealed that the retina may exert control over deep brain function and may be importantly involved in the etiology, progression, and treatment of disorders such as Parkinson's disease (PD). While such a concept is uncharted territory and even less is known about the mechanism by which this might be achieved, this study was undertaken to determine how retinal dopamine (DA), serotonin (5-HT), and melatonin (MEL) neurotransmitter systems might be involved in the control of movement in their own right. To explore these further, intravitreal (IVIT) injections of DA, 5-HT, and MEL were made 0.5 or 3 h prior to testing horizontal and vertical movement in the open field as well as assessment on three motor tests used routinely to evaluate movement as a preclinical model of PD. The doses of DA (2 µl of 25 and 75 µg/µl), 5-HT (2 µl of 5 and 15 µg/µl), and MEL (2 µl of 5 µg/µl) were chosen because of previous work demonstrating an anatomically precise effect of these transmitters after they were injected directly into the brain. The postinjection times of testing were also chosen on the basis of previous intracerebral and IVIT work intimating the importance of the circadian cycle in determining the efficacy of such effects. 0.5 h after IVIT injection of DA at the 25 and 75 µg/µl doses, significant inhibition of motor function was observed. While IVIT injection of 10 or 30 µg of 5-HT also inhibited motor performance, this was significantly less than that seen with DA. In fact, IVIT injection increases motor performance compared to vehicle injection on some parameters. The IVIT injection of 10 µg of MEL facilitated motor function on many parameters compared to DA, 5-HT, and vehicle injection. When rats were tested 3 h after IVIT injection, the inhibition of vertical movement was also observed compared to controls. The present results illustrate that specific retinal neurotransmitter systems participate in the normal control of bodily motor function. The possible involvement of these systems in movement disorders such as PD is the subject of ongoing research.

**Keywords: dopamine, serotonin, melatonin, retina, Parkinson's disease, intravitreal, motor function, microinjection**

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***Edited by:***
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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *11 January 2017*
***Accepted:*** *21 June 2017*
***Published:*** *05 July 2017*

***Citation:***
*Willis GL and Freelance CB (2017) Neurochemical Systems of the Retina Involved in the Control of Movement. Front. Neurol. 8:324. doi: 10.3389/fneur.2017.00324*

## INTRODUCTION

Recent evidence has emerged proposing that the retina is functionally linked to nigro-striatal dopamine (NSD) system control over motor function. This has been illustrated in a number of ways, including an intimately detailed proposition that there is a neurological system running between the retina and the pineal gland that communicates with crucial hypothalamic and midbrain structures during its course of passage (1). In addition, the simple pharmacological illustration that the intravitreal (IVIT) injection of microgram quantities of anti-Parkinsonian drugs can restore

impaired NSD system function, in a pattern emulating the course of circadian function, is an intriguing finding (2). In fact, the observed behavioral recovery occurs even though the variables of anatomical distance and compartmentalization of the retina render the two events anatomically unrelated. From a traditional perspective whereby impaired motor function of Parkinson's disease (PD) is reflexively attributed to impaired NSD system function, such a finding would be simply regarded as inexplicable. However, a deeper examination of both clinical and experimental manifestations of this disease reveal that impaired visual function is shared and that dopamine (DA) deficiency of the retina is, in fact, a bi-product of the disease as well (3–5). What remains a further mystery is why DA deficiency of the retina occurs in parallel with the development of PD and how these two events may be anatomically, physiologically, and etiologically linked. Indeed, such findings require a deeper examination as to the definition of PD, its etiology, and the role of the retina in movement. While the role of the NSD system in PD sits as the core proposition upon which the entire arsenal for treating this disease has been developed (6), this presents problems with interpretation since DA deficiency in the retina and NSD system occur concomitantly (3–5). At the very least, to imply cause and effect, good scientific procedure would dictate that retinal and NSD involvement would each have to be studied individually to determine their participation in the possible sequelae of events underlying this disorder.

Recent experimental and clinical reports have demonstrated retinal involvement in the etiology (7) and symptomatic improvement of PD (2). While such work has examined neurotransmitter function in the pathological state of DA deficiency, work on the role of retinal neurotransmitters in motor performance of non-PD rats with normal retinal function has not been examined. Therefore, it was hypothesized that if IVIT administration of neurotoxic (7) and therapeutic agents (2) can induce and repair experimental PD by their effect upon the retina, then the IVIT administration of neurotransmitters should also induce change in gross motor function in normal animals. Given that DA, serotonin (5-HT), and melatonin (MEL) occur naturally in mammalian retina (8–15), these neuroactive substances were selected for testing in this study and were injected in doses so minute that if any alteration in motor performance was observed then such changes could not be attributed to diffusion of the drug into CNS sites known to be involved in motor control (16–18).

## MATERIALS AND METHODS

42 male, Sprague-Dawley rats were obtained from the Bronowski Institute colony and were housed individually in plastic boxes with wire mesh tops. Standard food pellets (Clarke King®/ Barastock®) were made available *ad lib* from a feeding grid on the top of each cage while tap water was also made available through the feeding grid. Animals ranged in weight from 250 to 350 g at the commencement of the experiment. Room temperature was maintained at 22°C ± 2° with a 12 h light: 12 h dark cycle with lights on at 0700 h. The room was illuminated with two fluorescent tubes with the intensity of light within each cage averaging 250 lx during the light phase of the light/dark cycle. No light was detected in the housing facility during the dark phase. This study was carried out in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes and was approved and monitored by the Animal Experimentation Ethics Committee of the Bronowski Institute of Behavioral Neuroscience.

### Study Design

#### Study I: IVIT DA

After habituation into the colony for at least 7 days, all animals were handled by all experimenters prior to commencing the formal part of each study to minimize stress. Prior to determining the effects of the test drugs or vehicle solutions on motor performance, all rats underwent control measurement at least 6 days prior to drug testing and these served as the baseline measurements for all DA experiments. In the *first experiment*, two groups of rats consisting of seven animals per group were employed with the first group receiving a bilateral IVIT DA (50 µg in 2 µl) injection, while the second group received a 2 µl IVIT injection of vehicle, bilaterally. These rats were tested 30 min after injection just prior to assessment of motor function during the light phase of the light/dark cycle (see Behavioral Measures). For dark phase testing, all animals were injected again with either DA (50 µg in 2 µl) or vehicle (2 µl) and then tested on all motor tests allowing at least 48 h between injections. Rats were then given 6 days to recover from the first study and then the same paradigm was repeated for the *second experiment* using the same animals, except that 3 h was permitted between the time of injection and the time of assessment of motor function. The animals were again used in a *third experiment* following the same paradigm as described for the first study with the exception that a dose of 150 µg in 2 µl (bilaterally) was employed for the IVIT injection. As with previous studies, 48 h elapsed between light phase and dark phase testing while the amount of rest time between the second and third experiments was 6 days.

#### Study II: IVIT 5-HT

In a *fourth experiment*, 14 more rats were tested for baseline performance and then divided into two groups of rats with seven animals per group. One group was injected bilaterally with IVIT 5-HT (10 µg in 2 µl), while the second group of rats were injected with 2 µl of vehicle. As with Study I, motor function was again examined 30 min after IVIT injection during the light phase and dark phase testing, allowing 2 days of recovery before performing the dark phase test. The same rats were used in a *fifth experiment* that was procedurally identical with experiment four. All parameters were the same with the exception that 5-HT was used for IVIT injection in a dose of 30 µg in 2 µl (bilaterally) during the light and dark phases of the L/D cycle.

#### Study III: IVIT MEL

In the *sixth experiment*, a new group of seven animals were given a bilateral IVIT MEL (10 µg in 2 µl) injection, while a second group of seven received the control bilateral 2 µl IVIT vehicle injection. The paradigm applied was identical to experiment five with all

rats tested 0.5 h after IVIT injection during both the light phase and the dark phase of the L/D cycle.

## Drugs and Solutions for IVIT Injections

Dopamine hydrochloride was acquired from Sigma-Aldrich (St. Louis, MO, USA; product no. H8502) and was prepared for IVIT injection at concentrations of 50 and 150 μg/2 μl by dissolving it in 0.09% sterile saline solution (Pfizer). 5-HT creatinine sulfate complex (Sigma Chemicals; product no. H7752) and mixed in the concentrations of 10 and 30 μg/2 μl and dissolved in 0.09% isotonic saline. MEL (Sigma-Aldrich; product no. M5250) was prepared at the concentrations of 10 and 30 μg/2 μl in 75% solution of dimethyl sulfoxide (DMSO). Vehicle injections for all other drugs were made with the 0.09% saline solution with the exception of MEL where 2 μl of a 75% DMSO solution was employed. New solutions of drug were prepared immediately prior to each injection and were kept on ice and protected from light and were discarded immediately at the end of each injection session. All concentrations of drug employed in both studies were chosen on the basis of previous work describing a localized effect of these compounds when injected by the intracerebral route (19–23). In consideration of the dose, volume of injectate and potency of effect from previous studies, any findings in this study would preclude the possibility that they represent the effect of a leakage of test substances into sensitive brain areas after IVIT administration.

## IVIT Injections

Injections into the vitreous humor were made with the aid of a 10 μl syringe fitted with a 26 g needle 75 mm in length. The needle was fitted with a colored plastic sleeve exposing 3 mm of the tip to allow the experimenter to gage the depth of needle insertion into the center of the vitreal mass. Rats were first placed in a clear Perspex induction chamber (200 mm × 300 mm × 400 mm) fitted with a base constructed from heavy gage plastic netting above a 25 mm layer of cotton baton that served to absorb and hold the anesthetic. Isoflurane inhalation anesthetic (Attane-Bomac: 1 mg/ml) was employed by placing approximately 10–20 ml into the absorbent cotton surface just prior to placing the animal into the induction chamber. Exposure of the animal for 60–100 s induced a state of deep anesthesia that lasted 60–90 s, thereby permitting the injection of the test substances and vehicle bilaterally into the vitreous. To keep the preparation clean, the surrounding fur was swabbed with ethanol prior to injection. To facilitate injection into the lateral aspect of the eye, light pressure was placed on the caudal surface of the eye using a sterile, gloved, tip of the index finger to cause it to become a maneuverable exophthalmic mass and thereby permit the experimenter to gently apply counter pressure when the needle was inserted into the vitreous mass. In each case, the appropriate drug was injected bilaterally in a volume of 2 μl, commencing with the left eye. Postinjection, the area was gently swabbed with sterile isotonic saline and a drop of antibiotic ointment [Amacin® eye and ear ointment (Jurox Pty Ltd., Rutherford, NSW, Australia)] was placed on the cornea as a prophylactic. Rats were held and kept warm until they were able to ambulate on their own before being returned to their individual cage.

## Behavioral Measures

Independent variables were measured in the same animals during the light phase and the dark phase of the L/D cycle commencing between 1000–1500 hours and again at 2000–0100 hours, respectively. For each drug dose tested, the effects of IVIT drug administration on motor performance were tested in the sequence of light phase testing followed by two rest days. If more than one dose of a drug was tested, then rats were rested for at least 6 days before subsequent testing commenced. This paradigm has been implemented previously (2, 17, 20, 22) as it reliably depicts the relationship in movement during the light phase versus the dark phase of the L/D cycle and is indicative of normal circadian patterns in this species.

Locomotion (horizontal movement) and rearing (vertical movement) were measured with the aid of a 900 mm (length) × 500 mm (width) × 300 mm (height) PVC box fitted with machine vision with motion detection capabilities as implemented previously (2, 7, 24, 25). The number of movements within the horizontal plane and the number of rearing-associated movements in the vertical plane during each 10-min test session were measured and recorded with the aid of specialized software. A series of three motor reflex tests were performed immediately at the conclusion of the open field test (24). These tests included the latency to retract the left and right front limbs when they were elevated 25 mm from the table surface, the latency to step up or down from a raised platform when the rear torso was elevated 25 mm and the latency to ambulate outside of a 90 mm × 170 mm rectangle. These tests are derivations of those described originally by Balagura et al. (26) and have been used extensively to characterize the features of experimental PD (24). The test chamber and all surfaces and each apparatus were thoroughly washed between the testing of each animal to avoid scent contamination that may cause distraction during testing. Testing during the dark phase of the light/dark cycle was performed under low intensity red light with all sources of illumination masked by implementing red barrier filters (Lee Filters no. 106 "Primary Red").

## Statistical Analysis

Due to the high degree of between-subject variability that commonly exists in horizontal and vertical movements, different scores were calculated by subtracting performance after IVIT injection with baseline performance during control measurements taken prior to commencing the formal study. The latency to retract, step, and ambulate in drug- and vehicle-treated groups were analyzed as raw scores. A one-way analysis of variance (ANOVA) with *post hoc* LSD was first employed to determine whether there was a main effect across all drug-treated animals at 30 min post IVIT injection for each parameter examined for the light phase and the dark phase of the light/dark cycle. The Levene test for homogeneity of variance was performed and, if significant, the Games–Howell (GH) *post hoc* test was implemented assuming inequality of variance. A one-way ANOVA was also used to examine differences between each drug and vehicle-treated animals tested 3 h after the IVIT injection. If Levene's test returned as statistically significant, then nonparametric independent samples Mann–Whitney *U* (MWU)

test was employed for comparing each drug-injected group to their vehicle-injected counterparts. Given that the hypothesis permitted prediction of the direction of the expected outcome, a one-tailed test was employed with exact significance. The confidence levels were chosen *a priori* and set at 5% to depict a significant effect, while *p*-values ranging from 0.051 to 0.099 depicted a significant trend. All vehicle-/saline-injected controls for each parameter for all animals tested 30 min after IVIT injection were combined after analysis revealing that they did not differ prior to commencing IVIT drug testing. Analysis of control performance after IVIT administration of 75% DMSO was not significantly different to that of rats receiving IVIT control injection of isotonic saline. This permitted the combining of all control sessions into one group for statistical comparison across all drug-treated groups tested at 30 min post IVIT injection. All statistical analyses were performed using SPSS Statistics v.24 (IBM, Armonk, NY, USA).

## RESULTS

As shown in **Figure 1A**, there was a significant impairment of horizontal movement during the light phase of the L/D cycle in rats receiving IVIT injection of 50 μg of DA compared to rats receiving IVIT injections of isotonic saline [ANOVA (main effect): df = 5.69; $F = 3.117$, $p = 0.014$; *post hoc* LSD multiple comparisons: $p = 0.001$]. The 50 μg DA injection group was also more impaired than the 10 and 30 μg 5-HT groups ($p = 0.006$ and $p = 0.022$, respectively) as well as the 10 μg of MEL ($p = 0.002$). A weak, significant trend of improvement was also seen after 10 μg of MEL compared to 150 μg of DA ($p = 0.081$).

**Figure 1B** illustrates the changes in horizontal movement during the dark phase of the L/D cycle after IVIT injections of DA. While a significant main effect was not observed (ANOVA: df = 5.69; $F = 1.345$, $p = 0.257$), *post hoc* LSD analysis revealed that rats injected with the 50 or 150 μg doses of DA differed significantly from those injected with 10 μg of MEL, as they were less impaired ($p = 0.043$ and $p = 0.048$, respectively). There was also a strong trend for improvement after 10 μg of MEL compared to the control group ($p = 0.067$).

The effect of IVIT injections on vertical movement during the light phase are expressed in **Figure 2A**. ANOVA revealed a significant main effect (df = 5.69; $F = 10.062$, $p < 0.001$), with LSD *post hoc* comparisons illustrating significant differences between 50 μg DA compared to control injection ($p < 0.001$), 10 μg of 5-HT ($p = 0.002$), 30 μg of 5-HT ($p = 0.024$), and 10 μg of MEL ($p < 0.001$). The 150 μg DA group tested at 30 min were also found to be more severely impaired on this parameter compared to controls ($p < 0.001$), 10 μg of 5-HT ($p = 0.032$), and the 10 μg of MEL groups ($p < 0.001$). Rats injected with 30 μg of 5-HT showed significantly less vertical movement during the light phase when compared to controls ($p = 0.027$) and those injected with 10 μg of MEL ($p = 0.002$). Rats injected with 30 μg of DA and tested at 3 h were significantly impaired at that time compared to their saline-injected counterparts ($p = 0.021$). There was also a strong trend for MEL-injected rats to display improved motor performance compared to saline-injected controls ($p = 0.052$).

Intravitreal injections of various compounds during the dark phase of the L/D cycle (**Figure 2B**) were without effect on vertical movement, with the exception of the MEL-injected group. With the main effect showing a high level of significance (ANOVA: df = 5.69, $F = 3.628$, $p = 0.006$), robust decreases were observed between MEL and the following groups: control ($p = 0.016$), 50 μg DA ($p < 0.026$), 10 μg of 5-HT ($p = 0.016$), and 30 μg of 5-HT ($p = 0.004$) (all *p* values were derived from *post hoc* GH analysis assuming unequal variance: Levene Statistic, $p = 0.012$).

The effect of IVIT injections on latency to retract a limb were virtually without effect during both the light and dark phases of the L/D cycle. ANOVA revealed non-significant effects (**Figure 3A**: light phase: df = 3.139; $F = 1.023$, $p = 0.407$; **Figure 3B**: dark phase: df = 5.139, $F = 0.801$, $p = 0.551$). The only significant difference between groups in the ability to retract a limb during the light phase was after the IVIT injection of 10 μg of 5-HT compared to controls ($p = 0.039$, *post hoc* GH analysis assuming unequal variance: Levene Statistic, $p = 0.028$).

As shown in **Figure 4A**, there was no significant change in the ability to step up or down from a raised platform during the light phase of the L/D cycle in any of the drug-treated groups [ANOVA (main effect): df = 3.41, $F = 0.447$, $p = 0.721$], nor as revealed with the *post hoc* LSD analysis. However, during the dark phase (**Figure 4B**), a weak significant trend was revealed (ANOVA: df = 5.69, $F = 2.193$, $p = 0.066$). *Post hoc* GH analysis revealed a weak trend of improvement in the ability to step down for 10 μg 5-HT versus controls ($p = 0.098$), with a Levene Statistic of $p < 0.001$.

**Figure 5A** illustrates that there was a trend to increase the latency to ambulate after IVIT injection during the light phase (ANOVA: df = 5.69, $F = 1.778$, $p = 0.130$). On *post hoc* analysis, the group receiving 150 μg DA was significantly slower than those receiving 10 μg of 5-HT ($p = 0.018$), 30 μg of 5-HT ($p = 0.015$), or 10 μg of MEL ($p = 0.031$). A strong trend was also revealed, with 150 μg DA being slower than controls ($p = 0.055$). During the dark phase (**Figure 5B**), no significant main effect was detected (ANOVA: df = 5.69, $F = 1.812$, $p = 0.123$), while there was no difference between any of the drug-treated groups as revealed by *post hoc* GH analysis (Levene Statistic $p < 0.001$).

For the rats measured 3 h after receiving an IVIT injection during the light phase, only limb retraction was significantly faster in rats injected with 50 μg DA (ANOVA: df = 1.13, $F = 6.998$, $p = 0.021$) compared to controls. Furthermore, a significant, weak trend toward slowing the ability to step down during the dark phase was also seen in these rats treated with 150 μg DA 3 h prior to testing (Levene's test for homogeneity $p = 0.005$, MWU test $p = 0.097$).

## DISCUSSION

The present results demonstrate that the injection of minute quantities of DA, 5-HT, and MEL into the vitreous can alter horizontal and vertical movement and motor reflex control. These substances were chosen for study on the basis of their functional importance in the retina as well as their role in normal motor function and in pathological conditions such as PD (8–15). Furthermore, this study is an important advance on

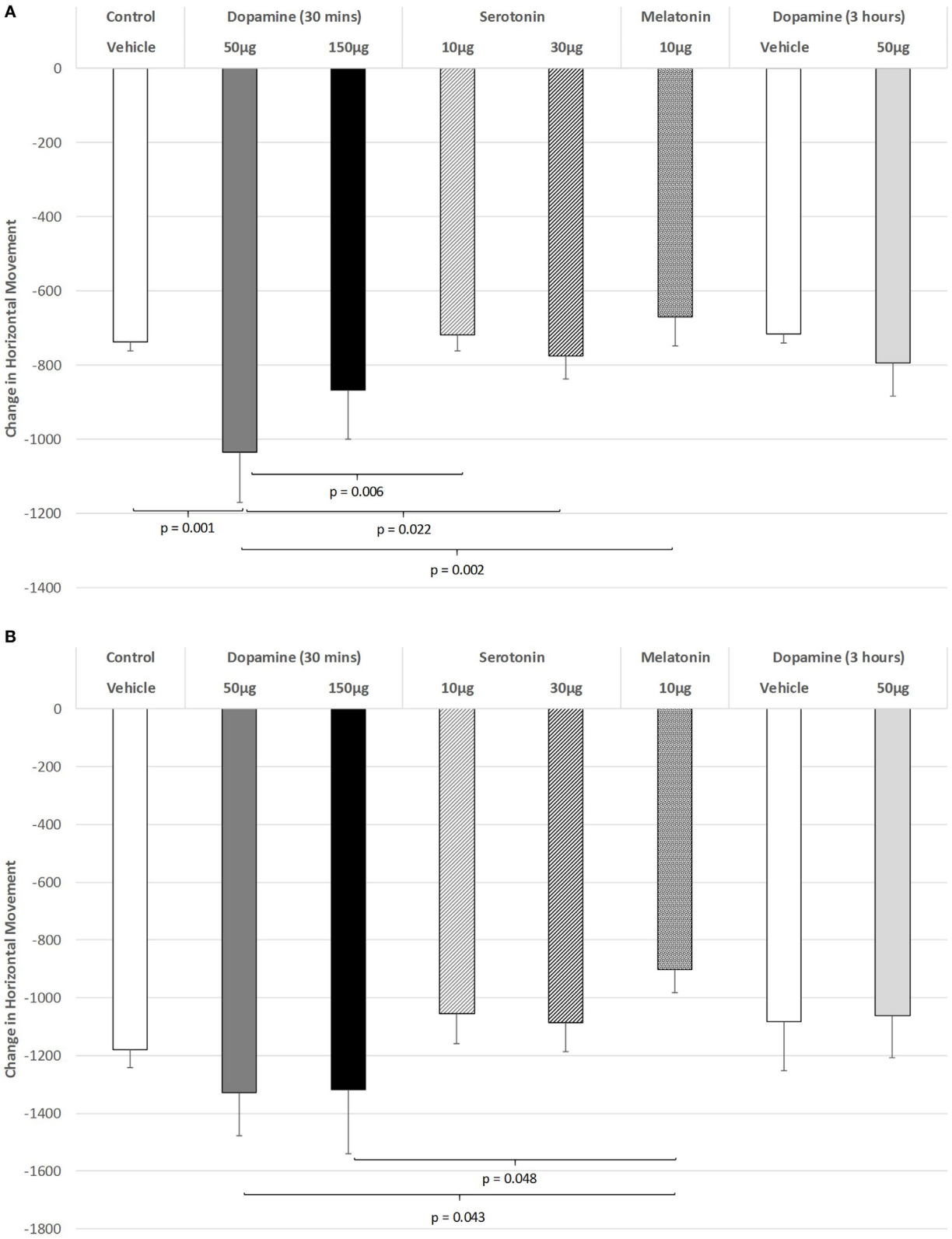


**FIGURE 1** | The effect of intravitreal (IVIT) dopamine (DA), serotonin, melatonin, and saline (vehicle) on horizontal movement during the **(A)** light phase and **(B)** dark phase of the light/dark cycle. Difference scores were obtained by subtracting the drug injection performance from the non-injected control performance. Rats were tested 30 min after IVIT injection in all groups with the exception of an additional group receiving 2 µl of control solution bilaterally and a second group receiving 50 µg of DA 3 h before testing. Values in all control animals tested 0.5 h after injection were combined into a single control group as they did not differ significantly from each other. The T-bars represent the SEM.

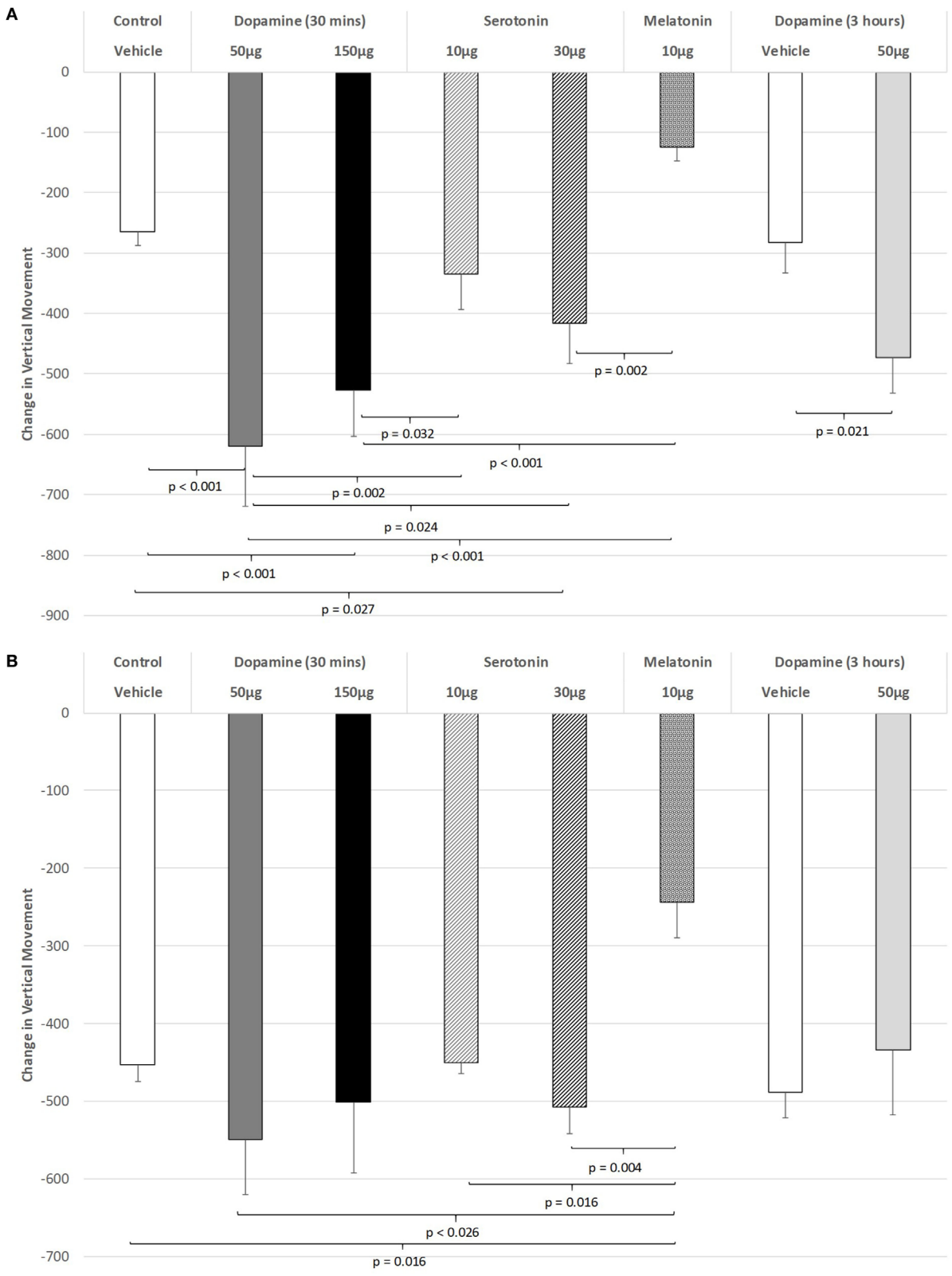


**FIGURE 2** | The effect of intravitreal (IVIT) dopamine (DA), serotonin, melatonin, and saline (vehicle) on vertical movement during the **(A)** light phase and **(B)** dark phase of the light/dark cycle. Difference scores were obtained by subtracting the drug injection performance from the non-injected control performance. Rats were tested 30 min after IVIT injection in all groups with the exception of an additional group receiving 2 µl of control solution bilaterally and a second group receiving 50 µg of DA 3 h before testing. Values in all control animals tested 0.5 h after injection were combined into a single control group as they did not differ significantly from each other. The T-bars represent the SEM.

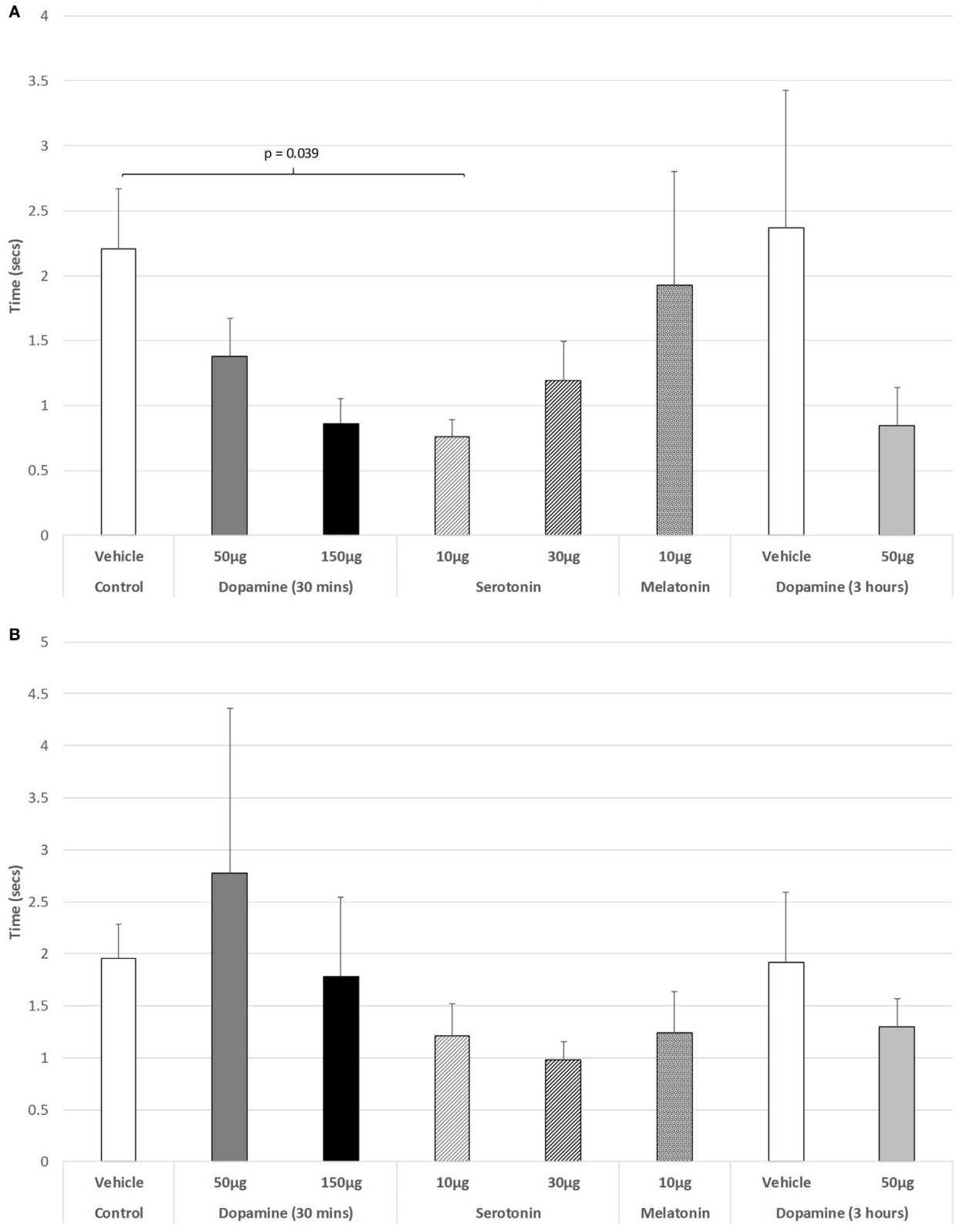


**FIGURE 3** | The effect of intravitreal (IVIT) dopamine (DA), serotonin, melatonin, and saline (vehicle) on latency to retract a limb during the **(A)** light phase and **(B)** dark phase of the light/dark cycle. Rats were tested 30 min after IVIT injection in all groups with the exception of an additional group receiving 2 µl of control solution bilaterally and a second group receiving 50 µg of DA 3 h before testing. Values in all control animals tested 0.5 h after injection were combined into a single control group as they did not differ significantly from each other. The T-bars represent the SEM.

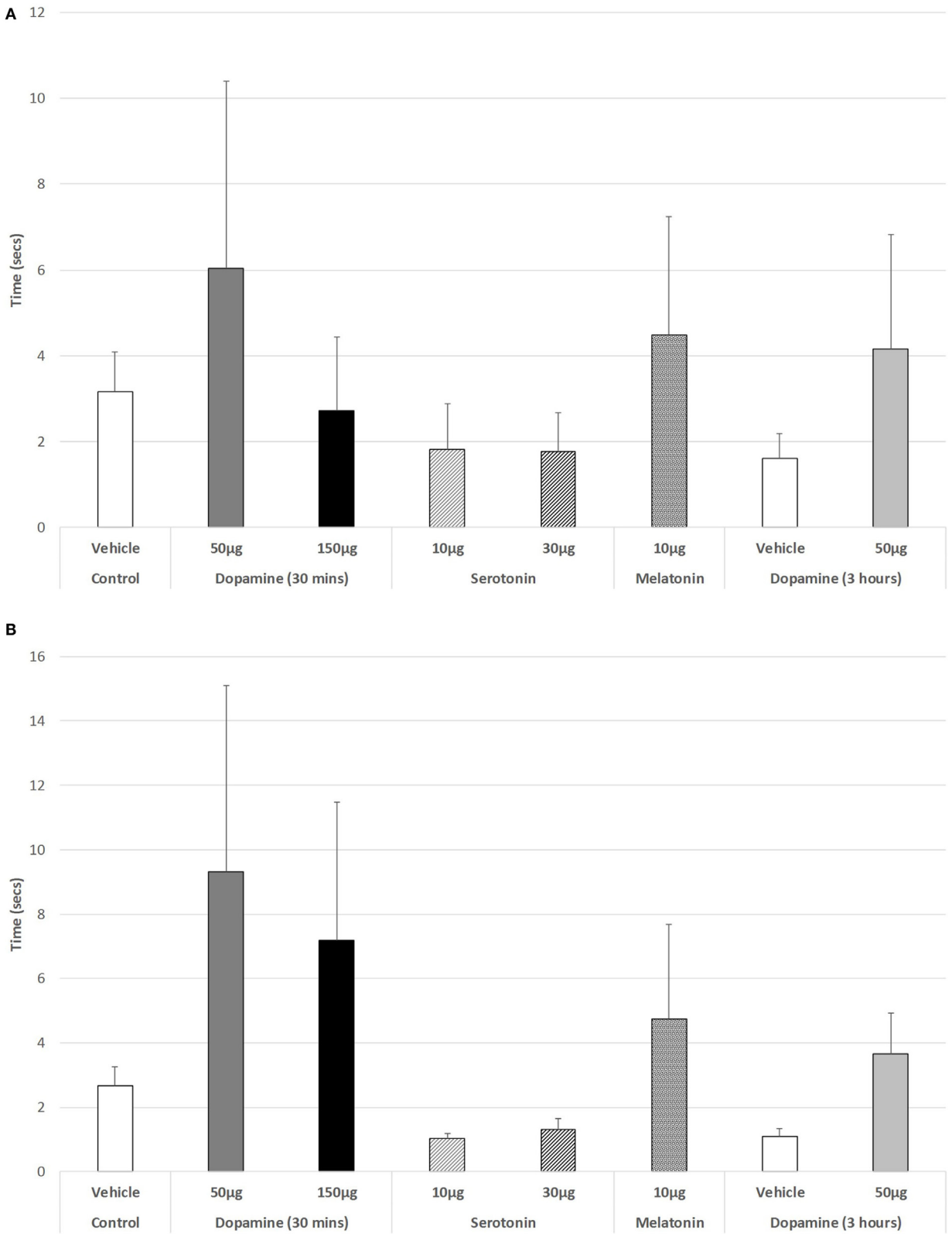


**FIGURE 4** | The effect of intravitreal (IVIT) dopamine (DA), serotonin, melatonin, and saline (vehicle) on latency to step up or down during the **(A)** light phase and **(B)** dark phase of the light/dark cycle. Rats were tested 30 min after IVIT injection in all groups with the exception of an additional group receiving 2 µl of control solution bilaterally and a second group receiving 50 µg of DA 3 h before testing. Values in all control animals tested 0.5 h after injection were combined into a single control group as they did not differ significantly from each other. The T-bars represent the SEM.

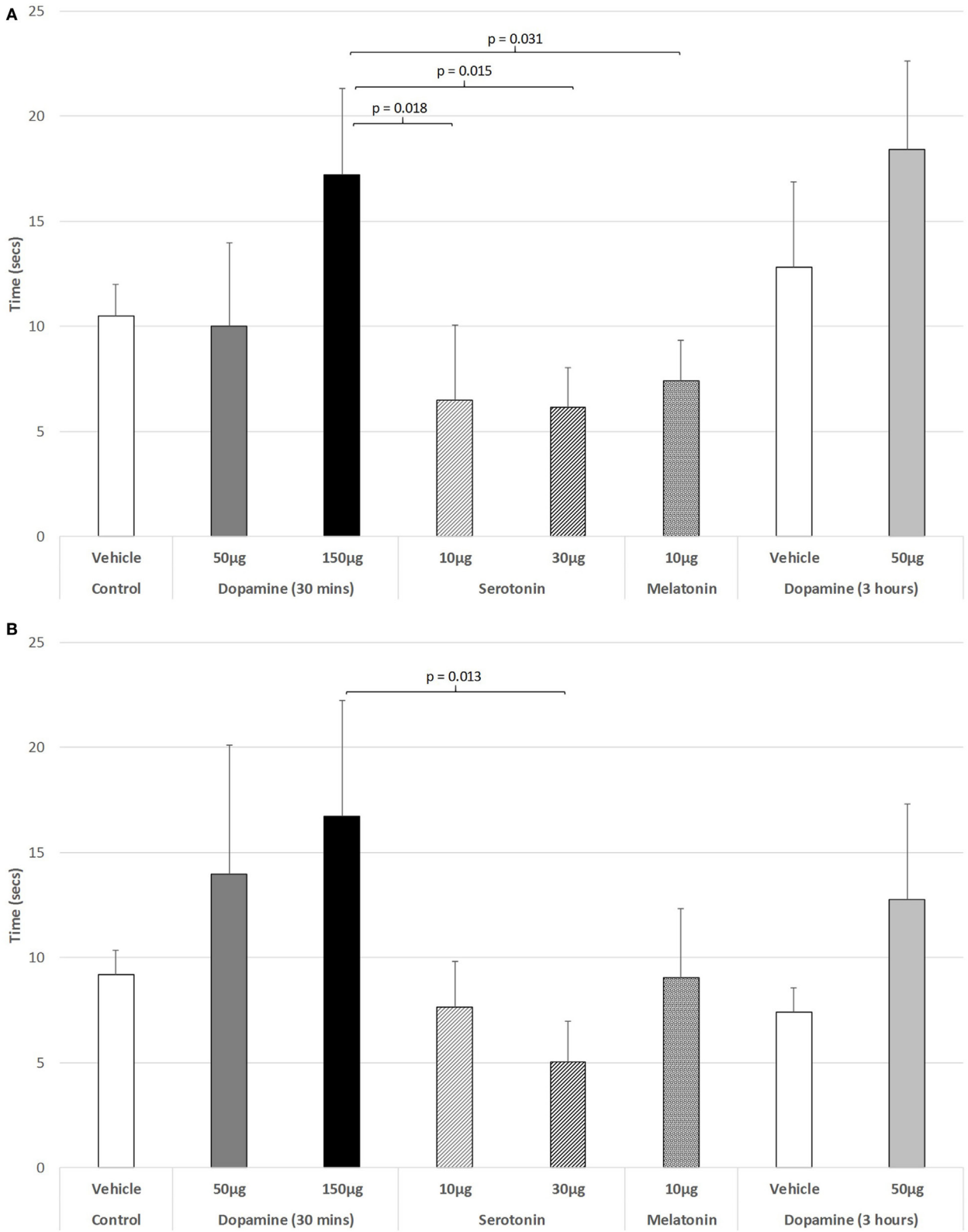


**FIGURE 5** | The effect of intravitreal (IVIT) dopamine (DA), serotonin, melatonin, and saline (vehicle) on latency to ambulate from a prescribed area during the **(A)** light phase and **(B)** dark phase of the light/dark cycle. Rats were tested 30 min after IVIT injection in all groups with the exception of an additional group receiving 2 µl of control solution bilaterally and a second group receiving 50 µg of DA 3 h before testing. Values in all control animals tested 0.5 h after injection were combined into a single control group as they did not differ significantly from each other. The T-bars represent the SEM.

previous work whereby IVIT administration of minute quantities of the DA precursor L-DOPA and the MEL receptor antagonist ML-23 significantly repaired experimental PD (2). In our attempt to examine the role of the retina as the initiatory locus for light to stimulate the circadian system (27, 28), the present findings illustrate the importance of retinal systems in exerting control over deep brain modulation of motor function. That the dose of drug employed in this study is rendering its effect directly upon the retina is intimated by two lines of evidence. First, the dose of drugs are so low that when similar, or even larger, doses are injected directly into brain sites suspected to have an effect on various physiological and behavioral parameters, no effect is seen when such injections are translocated only a few millimeters away (18). This is an important concept given that the distance from brain sites actively involved in motor control in the rat are not only located 25 mm away from the retina, but they are also contained within a separate body compartment. Second, recent studies illustrated that herbicides, insecticides, and neurotoxins that were injected into the vitreous of the rat can induce a state closely resembling experimental PD (7). The doses of such toxins, when injected directly into the brains of this species, have no effect when placed 2 or 3 mm from the intended anatomical target (i.e., the NSD system). These published results also dovetail with the present findings to suggest which neurochemical systems in the retina may be involved. From this, we draw the tentative conclusion that the retina is the location where anti-Parkinsonian medication might be rendering at least some of its therapeutic effect or even inducing adverse side effects.

Given the exploratory nature of this study, the underlying hypothesis would be bidirectional in predicting outcome. This is based on previous work concerning the role of circadian, NSD, and retina DA systems in movement demonstrating that the ability of DA to provide therapeutic relief or to enhance motor impairment in PD is dependent upon: the dose and type of DA replacement administered, the anatomical site involved, and the motor parameter examined. For example, inhibition of motor performance has been reported in experimental PD after systemic L-DOPA and other forms of DA replacement (29). This effect is seen in experimental models of PD, in intact rats and in rats with compromised circadian function (1, 30). Clinically, L-DOPA has been shown to exacerbate motor impairment, cause dysphonia, impair swallowing, aggravate depression, and enhance tremor in PD patients (31–40). Of the substances and doses tested, DA in both doses caused more inhibition of motor performance during the light phase than the dark phase of the L/D cycle and was never effective in reversing the deficits in motor performance at either of the two doses employed but caused them only to worsen. While 5-HT did not cause such inhibition of motor performance, the effect of 5-HT in either dose was not as inhibitory as DA. Motor performance after MEL was better during the dark phase than the light phase of the L/D cycle, with this dual effect between DA and MEL being similar to that seen in previous studies (2). This suggests participation of retina as part of circadian system in the control of deep brain modulation of motor function. The present and previous results (1) reveal a complex system regulating motor performance in the normal and pathological brain, intimating that the effect of therapeutic drugs used for treating PD will now have to be screened for their effects upon the retina as well as their ability to replace deficient DA in the NSD system. Further research will help to reveal the participation of each in the neurological matrix governing motor control in vertebrates.

One of the principal reasons for undertaking this study was to determine the role of the retina, as the initiatory site of the response to light, in the circadian system in motor function. To achieve this, injections were made prior to testing during the light or dark phase of the L/D cycle and the difference in response between the light phase and the dark phase was remarkable. For example, the inhibitory effect of DA on motor function when compared to 5-HT or MEL was much more potent during the light phase than during the dark phase. MEL, on the other hand, was more effective at improving horizontal and vertical movements during the light phase and the dark phase, but was particularly effective during the light phase for vertical movement. This is consistent with the circadian-related therapeutic effects that were observed when MEL antagonism was achieved *via* the IVIT administration of MEL receptor antagonist ML-23 during the dark phase, while this effect was less potent when applied during the light phase (41, 42). Similarly, when L-DOPA was administered *via* the IVIT route, it was more effective when administered during the light phase compared to the dark phase (2). The previous IVIT administration of neurotoxins showed similar light/dark differentiation (7), providing further support for the hypothesis that the circadian system is involved in the etiology of PD (7, 43). Traditional clinical work with PD supports the contention of circadian involvement, in that symptom expression varied with changes in the L/D cycle, as well as the increasing interest in the use of light treatment in this disorder (27, 28, 44, 45).

While differences in light phase versus dark phase were consistent with other studies and are understandable in relation to results from previous studies, the effect of IVIT injections on some motor parameters (i.e., horizontal and vertical movements, and ambulation) while others parameters (i.e., latency to retract and to step) were not affected reveals a differential sensitivity of the systems involved in different aspects of motor control. In previous work (7), we have found that latency to retract a limb is a particularly sensitive motor measure compared to other measures, such as stepping or latency to ambulate. Why this showed no significant changes across the different conditions in this study is yet to be explained. However, the present studies are pioneering in that the doses employed are extremely low in comparison to those needed to evoke a response after systemic or even intracerebral doses commonly employed. Future studies examining dose dependency may reveal which systems are involved and their relative sensitivity to the applied doses, that may help to differentiate which systems control what aspects of motor function in more detail.

It is interesting to note that the neurotransmitters and hormone studied in this study do not appear to render an effect on motor function as potent as those achieved previously with the precursor L-DOPA (2) or the MEL receptor antagonist ML-23 (41, 42). While this might represent a potency problem that may be resolved with further dose–response studies, it might alternatively suggest that these could represent more effective means acting on other mechanisms for treating the disease, combined

with a more effective route of administration. This is consistent with the explanation as to why L-DOPA remains the most widely used therapeutic for PD (6). In addition, PD patients are characterized as having comorbid problems with sight (3, 4, 46, 47) further suggesting that the visual system is, in fact, mediating the observed effects of L-DOPA therapy. It is interesting to note further that when PD patients are administered anti-PD drugs, it is often reported that their vision also improves with such treatment (48). When considering the relationship between the retina and deep brain, it is worth bearing in mind that the retina is the only sensory organ that embryologically emerges from brain, whereas all other sensory systems are derived from cells that extend connections into the brain. While pharmaceutical companies spend extensively on studies devoted to "targeting" anatomical sites in the CNS that are candidates for treatment intervention, this study confirms previous findings suggesting that in PD, the retina provides direct access to deep brain function and in particular motor control. Results from this study support the suggestion that the retina is the site from which PD may commence and progress (2) and ultimately it is a locus from which it might be treated. By this line of reasoning, a more causal relationship between retinal function and PD is evident and from this emerges a novel hypothesis that needs to be more fully explored. The implementation of IVIT injections to target retinal systems with minute drug doses may serve to dramatically redefine the effective therapeutic dose and reduce side effects of DA replacement. With this, the need for more complex and invasive procedures that detract from the quality of life in PD patients would be diminished.

## ETHICS STATEMENT

This study was carried out in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes. The protocol was approved and the experiment monitored by the Animal Experimentation Ethics Committee of The Bronowski Institute of Behavioural Neuroscience.

## AUTHOR CONTRIBUTIONS

GW was responsible for design and conception of the work. GW and CF were responsible for acquisition, analysis, and interpretation of the data and for preparing the manuscript.

## ACKNOWLEDGMENTS

The authors wish to thank Cleo Moore and Monica Dumitrescu for technical assistance.

## FUNDING

This research was funded internally by The Bronowski Institute of Behavioural Neuroscience.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 16 August 2017
doi: 10.3389/fneur.2017.00389

# Visual Dysfunction in Posterior Cortical Atrophy

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Posterior cortical atrophy (PCA) is a syndromic diagnosis. It is characterized by progressive impairment of higher (cortical) visual function with imaging evidence of degeneration affecting the occipital, parietal, and posterior temporal lobes bilaterally. Most cases will prove to have Alzheimer pathology. The aim of this review is to summarize the development of the concept of this disorder since it was first introduced. A critical discussion of the evolving diagnostic criteria is presented and the differential diagnosis with regard to the underlying pathology is reviewed. Emphasis is given to the visual dysfunction that defines the disorder, and the classical deficits, such as simultanagnosia and visual agnosia, as well as the more recently recognized visual field defects, are reviewed, along with the evidence on their neural correlates. The latest developments on the imaging of PCA are summarized, with special attention to its role on the differential diagnosis with related conditions.

**Keywords: posterior cortical atrophy, Alzheimer's disease (AD), Balint's syndrome, visual agnosia, visual fields, hemianopia, magnetic resonance imaging imaging**

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***Edited by:***
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***Specialty section:***
*This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology*

***Received:*** *11 April 2017*
***Accepted:*** *21 July 2017*
***Published:*** *16 August 2017*

***Citation:***
*Maia da Silva MN, Millington RS, Bridge H, James-Galton M and Plant GT (2017) Visual Dysfunction in Posterior Cortical Atrophy. Front. Neurol. 8:389. doi: 10.3389/fneur.2017.00389*

## INTRODUCTION

In 1988, Benson et al. described an intriguing progressive condition characterized by a complex visual disorder occurring in the absence of ocular dysfunction (1). Their title introduced the syndromic diagnosis "posterior cortical atrophy" (PCA) which has proven apt and has survived to the present. The most common deficits in their cohort were components of the Bálint (simultanagnosia, optic ataxia, and ocular apraxia) and Gerstmann (agraphia, acalculia, finger agnosia, and right-left disorientation) syndromes (2, 3); additional features were alexia, visual agnosia, and transcortical sensory aphasia. Eventually non-visual functions, such as language and memory, were affected but until a very late stage these deficits were relatively mild and the visual disorder remained the main source of impairment throughout the course of the disease. Insight was preserved until late. Neuroimaging of these patients indicated disproportionate volume loss in the posterior cortical regions, particularly in the occipital and posterior parietal lobes (1). This clinicoradiological syndrome was soon found to be associated with AD (4, 5), but, as opposed to the typical (amnestic) form of the disease, there was an anterior–posterior gradient in PCA, with the greater severity of change occurring in the occipital, parietal and posterior temporal lobes.

With an increasing number of cases coming to autopsy other pathological entities—such as corticobasal degeneration (CBD), Lewy body disease (LBD), and prion diseases (6, 7)—were occasionally described as the underlying cause of PCA. More recently, PCA was reported in an individual with a

mutation associated with frontotemporal dementia (8). AD, however, remains the single dominant cause, accounting for 62–100% of the cases in the largest cohorts (6, 7, 9). Thus, PCA has been recognized as one of the atypical variants of AD (10) and indeed it is occasionally referred to as the "visual variant" of AD but this implies a more certain pathological diagnosis than is usually the case. From an integrative perspective, it may also be understood within a continuum of phenotypic variation of AD, since considerable clinical overlap occurs between PCA and other AD variants, especially its amnestic and language presentations (11, 12), more so at later stages. With time all PCA patients will progress to dementia of the AD type. However, it is as a visual disorder that PCA gains singularity, for the visual deficits are usually extremely disabling, even when the patient may still be considered cognitively preserved. This characteristic implies that PCA patients differ from patients with more cognitive presentations of AD in several aspects, including diagnosis and management.

## EPIDEMIOLOGY

The prevalence of PCA is unknown. In clinical cohorts published by centers for cognitive disorders, it has been found to represent about 5% of the total AD cases (13, 14). No estimate is available from ophthalmic services. Age at diagnosis is mostly within the late 50s and early 60s (15–17) but PCA can affect individuals from the 40s (18) to the 80s (7). Females and males are equally represented in several studies (7, 17), but some have observed a female predominance (18–20).

Patients with PCA and their families will usually describe a time-consuming search for the diagnosis, including appointments with several specialists—usually optometrists and ophthalmologists—before a neurological disorder is suspected (21). It is not rare for patients to be provided with numerous pairs of spectacles or even undergo cataract surgery or other procedures only to learn later that the problem is not in the eyes. Given this experience, it is a common impression amongst specialists dealing with PCA patients that this condition is underdiagnosed.

## CLINICAL PROFILE

### Visual Manifestations

One of the difficulties in diagnosing patients with PCA is that, although they complain about problems with their vision, descriptions of their symptoms are often difficult for the non-specialist to analyze. They may just say they cannot see, describe their vision as blurry, or may refer to difficulties performing specific tasks such as driving or reading. Only a detailed examination may uncover the specific deficit(s) leading to functional impairment. In the following sections the most important visual deficits in PCA are described.

### Simultanagnosia

Simultanagnosia refers to the failure to perceive multiple visual locations simultaneously or to shift attention from one object to another, which results in a very restricted effective visual field (22). A patient may miss an object he or she has just seen or report that objects seem to appear or disappear from view. Simultanagnosia has been consistently demonstrated as the most frequent deficit in PCA, occurring in above 90% of the patients in several series (7, 15–17). It is a pervasive deficit that may be associated with some unusual behavior including the reverse-size phenomenon. This describes patients preferring to look at objects at distance, in order to appreciate them globally, or finding it easier to read small than large letters—such as the text rather than the headlines of a newspaper (23). In severe cases of simultanagnosia, perception of even a single, large object may be impaired, and sometimes an individual part of it may be mistaken for a different object [so-called "partonomic" error (24)].

Tasks relying on visual integration are used to test for simultanagnosia. Established tests include interpretation of a complex visual scene (25), such as the Boston cookie-theft picture, and reading fragmented letters. Failure to read the Ishihara pseudoisochromatic plates despite preserved color perception is a conspicuous feature in many patients with simultanagnosia (24, 26). The latter is often the only abnormality seen in the basic visual assessment of a PCA patient and its usefulness to raise the diagnostic suspicion cannot be overemphasized, although it is usually misinterpreted as a color deficit. However, the patients have as much trouble with the first (control) plate, which does not require color vision as do the subsequent plates. In its purest form simultanagnosia is considered due to impaired visual attention, which can be considered both in terms of shifts of attention to regions within the visual field and also shifts of attention related to the scale of the object to be processed. The former will be mirrored in impairment of ocular motor behavior but the latter may not be. However, it has also been argued that the attentional deficit may be object based resulting in failure to identify overlapping figures (objects at the same spatial location), or collocated objects where linking features have been weakened [such identifying correctly a star of David where the two component triangles are the same but not different colors (27)]. It should also be considered that the perception of illusory contours is a very early process in object identification and may occur as early as V2: this is likely related to the synthesis of partially occluded objects (28). Impairment of this early function in the identification of surfaces, which has been reported in simultanagnosia (29) would certainly seriously impair the identification of fragmented images.

### Other Elements of the Bálint Syndrome—Optic Ataxia and Ocular Apraxia

Simultanagnosia may occur in isolation or may be associated with optic ataxia and ocular apraxia, constituting the Bálint syndrome. Optic ataxia—lack of eye-hand coordination—refers to impaired reaching to objects when guided by vision with the preserved ability to do so when the object is accessed by means of other sensory modalities, e.g., sound (22), while ocular apraxia is a disorder of fixation, with the patient failing to fixate a specific object within the visual field in the absence of any ocular motor deficit (30). In PCA, the Bálint syndrome is often incomplete. Silmultanagnosia is thought to be an early finding, initially presenting in isolation or associated with ocular apraxia, with

optic ataxia developing later in the course of disease (17). Bálint syndrome is classically seen in the context of biparietal damage due to vascular disorders; however, it has been associated with PCA so often that its occurrence in a progressive manner should raise suspicion of the diagnosis.

## Visual Agnosia

Visual agnosia is a visuoperceptual disorder. It is defined as the inability to recognize objects presented visually, in the absence of any ocular or semantic deficit that could otherwise account for it (31). It is further divided into apperceptive and associative, according to the defective process being in the perceptual analysis of the object or in attributing a meaning to it, respectively. In PCA, the apperceptive subtype predominates (16, 32), demonstrated by the patient failing to copy a figure or match a figure with a sample (33). A particular form of visual agnosia affects the recognition of faces (prosopagnosia), a deficit that is a source of great social embarrassment. As with global visual agnosia, prosopagnosia in PCA is thought to be perceptual rather than agnosic in nature (17).

## Reading Disturbance

Trouble reading is one of the most frequent and disabling deficits for which PCA patients seek help. It can be due to acquired primary alexia (34), but most often reading impairment in PCA results from a combination of deficits including simultanagnosia, ocular apraxia, visual crowding (16, 24) and potentially homonymous visual field defects.

## Visual Field Defects

The occurrence of visual field defects in PCA has been a controversial subject, mainly due to the eloquence of the higher order visuospatial deficits that may arguably compromise the interpretation of visual field tests (35). Indeed, early PCA series dismissed visual field defects as exceptional in this condition (15). However, homonymous hemianopia or quadrantanopia was found in almost 50% of the patients in another series (7) and prevalence is even higher in groups of PCA patients who have visual fields performed as part of the workup (36–38), suggesting this deficit may be overlooked if not routinely searched for. Homonymous visual field defects are increasingly recognized as an early sign in PCA (39), and their occurrence may, remarkably, precede the higher order visual disorder (40, 41). **Figure 1** shows a typical visual field test result in a patient with PCA. The inferior quadrants are possibly more affected in the visual variant of AD (4, 42); this would imply involvement of the underlying optic radiations as occurs with bilateral occipitoparietal infarction, but this needs confirmation.

## Other Visual Deficits

Besides these major, well-characterized deficits, patients with PCA have been reported to complain of a variety of visual problems, including perceived motion of static stimuli, visual crowding, color washout, and prolonged color afterimages (23, 24). In addition, related deficits previously documented in AD patients and attributed to visual cortical pathology, e.g., abnormal contrast sensitivity and loss of color discrimination, particularly affecting short wave length (blue) stimuli (43), may apply to PCA as well. Basic visual skills, such as form detection and discrimination, color perception, and motion coherence were more recently highlighted as possibly contributing to the higher order visual deficits classically reported in PCA (19). A specific dysfunction of the magnocellular (M) pathway in AD, associated with impairment of motion perception and loss of achromatic contrast, has been proposed (44).

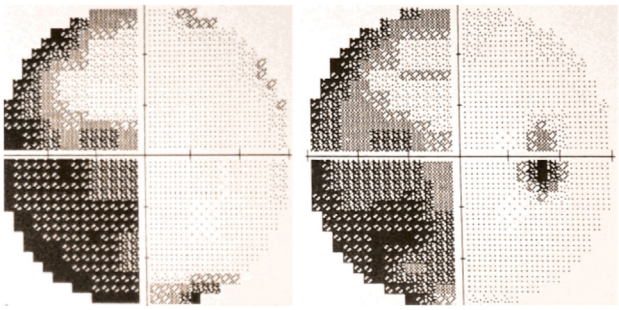

**FIGURE 1** | 30-2 Humphrey automated perimetry from a patient with posterior cortical atrophy (PCA) depicting left incomplete homonymous hemianopia. Published with the patient's authorization.

## Non-Visual Deficits

Non-visual deficits in PCA are mainly represented by disturbances of numeracy and literacy. These deficits may occur as part of a Gerstmann syndrome (agraphia, acalculia, left-right disorientation, and finger agnosia) or may be isolated. Attentional disorders may occur as visual (16, 45) or spatial neglect (46) or a combination of the two. Ideomotor apraxia is not rare, but it is usually mild in early stages, and if prominent should raise the suspicion of an alternative diagnosis, such as CBD. The same can be said if features of asymmetric parkinsonism are found. Polymini-myoclonus has been suggested to be an overlooked sign in PCA, present in a majority of patients (17). Visual hallucinations and REM-sleep behavior disorder have been occasionally observed in PCA, and are also thought to indicate an underlying non-AD pathology, namely DLB (17). Depression is common and is thought to be mainly reactional (35).

It should be noted that dressing and constructional apraxias, which are very common in PCA (7, 47), do not constitute apraxia in its proper definition, but rather visuospatial deficits (48).

# NEURAL CORRELATES OF THE VISUAL DEFICITS IN PCA

The higher order visual deficits observed in PCA patients are better understood as reflecting regional disruption of the two visual pathways for processing of object (ventral, "what" stream) and space (dorsal, "where" stream) (49, 50). This understanding has been favored by imaging studies showing correlation of simultanagnosia, the prototype of the visuospatial disorder in PCA, with greater atrophy in the dorsal (occipitoparietal) regions (20), while visuoperceptual deficits, e.g., visual agnosia, are associated

with predominant volume loss in the ventral (occipitotemporal) regions (34). Based on this schematic representation of visual processing, a clinical classification of PCA into a dorsal and a ventral subtype has been proposed (16). The first subtype would be represented by patients with predominant Bálint syndrome, apraxia, and neglect, while the second would be characterized by disproportionate visual agnosia, prosopagnosia, and alexia. A third subtype has been suggested to include patients with primary visual failure and impairment in basic visual skills (9, 51), in whom more marked occipital damage would be expected. Indeed, homonymous hemianopia was recently found to be associated with lateralized occipital degeneration in PCA (38). Interestingly, however, even in the presence of visual field deficits, the primary visual cortex remains relatively unaffected compared to higher visual areas.

In clinical practice, most patients present with a confluence of deficits relating to the occipital, parietal, and posterior temporal lobes, and this is mirrored by imaging studies showing considerable overlap of patterns of atrophy when these subgroups of patients are analyzed in combination (20).

## DIAGNOSIS

The critical element on the diagnosis of PCA clinico-radiological syndrome is the recognition of a progressive focal posterior cortical dysfunction associated with imaging evidence of damage to posterior cortical regions. As part of the clinical characterization, a formal neuropsychological assessment is necessary to establish the degree of involvement of individual cognitive domains and confirm that the disorder is relatively restricted to occipital and parietal regions.

### Diagnostic Criteria

Several clinical criteria for PCA have been published (7, 15, 16, 18), which are highly consistent in their definition of PCA. They all emphasize a higher visual disorder of insidious onset, manifesting with deficits of the dorsal and/or ventral stream, and which occurs with relative preservation of more anterior functions, such as memory and language. Some variability exists though in the delimitation of the syndrome. Remarkably, the Tang-Wai criteria (**Box 1**) introduce visual field defects as a core feature of PCA, given the same importance as simultanagnosia, constructional dyspraxia, environmental disorientation, and any element of the Gerstmann syndrome; at the same time, early parkinsonism and visual hallucinations, meant to distinguish LBD—a disease that may present with posterior cortical deficits but usually shows concomitant or fast developing involvement of other cortical and subcortical regions—are deemed exclusion criteria. Such phenotypic refinement may understandably impact on the specificity of these criteria.

Indeed, by excluding patients with specific LBD features, Tang-Wai et al. aimed to rule out LBD as a distinct condition from PCA; however, thereby, PCA caused by LBD or mixed (LBD-AD) pathologies are likely to be excluded as well (6, 7). Another conflicting issue is the inclusion of patients with PCA due CBD. Asymmetric parkinsonism and apraxia are suggested to distinguish PCA-CBD from PCA-AD, but these features are not addressed in a structured manner. In fact, if parkinsonism develops early in the course of disease, patients with PCA-CBD will be excluded. Therefore, while these criteria were not designed for the etiological diagnosis of PCA, they may be more specific for AD. On one hand, this characteristic has been critical for the very recognition of PCA as a variant of AD, on the other hand, a vacuum is left on how to classify those patients with a progressive posterior cortical dysfunction whose clinical features extend beyond the PCA typical phenotype.

A different (lumpers) approach to the diagnosis of PCA has recently been suggested (52). While preserving the core clinical description of the primary PCA syndrome (**Box 2**), the newly developed classification provides a solution on how to deal with atypical features, including them into a stratified framework to the etiological diagnosis (**Figure 2**): at the first level, the presence of the PCA clinico-radiological syndrome is established; the second level consists in of deciding whether PCA occurs in isolation (PCA-pure) or whether criteria for another neurodegenerative condition (e.g., visual hallucinations for LBD) are also met (PCA-plus); at the third level, these phenotypical categories are combined with the presence of pathology-specific biomarkers to yield a disease-level PCA description. Ideally, at this level, the patient will be given a diagnosis of PCA-AD, PCA-LBD, PCA-CBD, PCA-prion, and others. If a patient has PCA-pure and positive biomarkers for AD, the definitive diagnosis of PCA-AD may be given *in vivo*. However, since disease biomarkers are available only for AD and prion, and AD may manifest with such diverse phenotypes, diagnosis of PCA-LBD and PCA-CBD are currently presumptive and dependent on negativity to AD biomarkers. For instance, when patients fulfill criteria for both PCA and CBD and AD biomarkers are negative, the diagnosis of probable

**BOX 1 |** Diagnostic criteria for posterior cortical atrophy Tang-Wai et al. (7).

**Core features**

Insidious onset and gradual progression
Presentation of visual complaints in the absence of significant primary ocular disease explaining the symptoms
Relative preservation of anterograde memory and insight early in the disorder
Disabling visual impairment throughout the disorder
Absence of stroke or tumor
Absence of early parkinsonism and hallucinations
Any of the following findings:
- Simultanagnosia with or without optic ataxia or ocular apraxia
- Constructional dyspraxia
- Visual field defect
- Environmental disorientation
- Any of the elements of Gerstmann syndrome

**Supportive features**

Alexia
Presenile onset
Ideomotor or dressing apraxia
Prosopagnosia

**Investigations**

Neuropsychological deficits referable to parietal and/or occipital regions
Focal or asymmetric atrophy in parietal and/or occipital regions on structural imaging
Focal or asymmetric hypoperfusion/hypometabolism in parietal and/or occipital regions on functional imaging

**BOX 2 |** Core features of the posterior cortical atrophy clinicoradiological syndrome Crutch et al. (52).

**Clinical features:**
Insidious onset
Gradual progression
Prominent early disturbance of visual ± other posterior cognitive functions
**Cognitive features:**
At least three of the following must be present as early or presenting features ± evidence of their impact on activities of daily living:
- Space perception deficit
- Simultanagnosia
- Object perception deficit
- Constructional dyspraxia
- Environmental agnosia
- Oculomotor apraxia
- Dressing apraxia
- Optic ataxia
- Alexia
- Left/right disorientation
- Acalculia
- Limb apraxia (not limb-kinetic)
- Apperceptive prosopagnosia
- Agraphia
- Homonymous visual field defect
- Finger agnosia

**All the following must be evident:**
- Relatively spared anterograde memory function
- Relatively spared speech and non-visual language functions
- Relatively spared executive functions
- Relatively spared behavior and personality

**Neuroimaging:**
Predominant occipitoparietal or occipitotemporal atrophy/hypometabolism/hypoperfusion on magnetic resonance imaging/$^{18}$F-labeled fluorodeoxyglucose positron emission tomography/SPECT, single-photon emission computed tomography.
**Exclusion criteria:**
Evidence or a brain tumor or other mass lesion sufficient to explain the symptoms
Evidence of significant vascular disease including focal stroke sufficient to explain the symptoms
Evidence of afferent visual cause (e.g., optic nerve, chiasm, or tract)
Evidence of other identifiable causes for cognitive impairment (e.g., renal failure)

PCA-CBD may be appropriate. Alternatively, if a patient with PCA-plus tests positive for AD biomarkers, the diagnosis of AD may be given, although as disease-specific markers are not available for the second clinical suspected condition, the possibility of a dual pathology is still reasonable. These criteria take into account the suggested diagnostic criteria for AD (10) in their comprehensive approach of phenotype and disease-specific biomarkers. Their novelty is to include under the term PCA patients with posterior cortical dysfunction whom would otherwise not be given a unified diagnosis. Only further pathological studies will confirm the accuracy of these criteria. An expected consequence is an increment in the proportion of patients with non-AD pathologies in PCA cohorts. In the clinic the major priority is to recognize the syndrome and manage appropriately whatever the underlying pathology might be. Predictors of whether AD is the underlying pathology or not will not affect management materially until features of the other conditions are clinically apparent.

In the following sections, the imaging studies that support PCA as a clinico-radiological syndrome are reviewed, as well as *in vivo* pathological biomarkers for AD. In the particular case of LBD, to which no disease-specific biomarker is available, metabolic studies that are associated with the disease are also mentioned.

# IMAGING STUDIES

## Magnetic Resonance Imaging (MRI)

The syndrome of PCA can manifest without any detectable gray or white matter volume loss at MRI (24), but more commonly patients present with marked atrophy in the occpitoparietal and occipitotemporal regions bilaterally, but often more severe in the right hemisphere (15, 53, 54) (**Figure 3**). Although the disparate posterior volume loss is used to distinguish PCA from typical AD at imaging, it is not rare for patients with PCA to present coexisting atrophy in the mesial temporal regions, including the hippocampi (54). However, when directly compared, studies have generally shown only subtle differences in levels of atrophy between PCA and AD, with PCA patients showing greater atrophy in the right visual association cortex, while left hippocampal atrophy predominates in amnestic AD (53, 54). Millington et al. (38) used multimodal MR imaging to investigate structural and functional brain changes in a cohort of patients with PCA, all with visual field defects. Compared with healthy controls, cortical activation was reduced in the occipital lobes, with no significant lateralization, while gray matter loss was greater in extrastriate occipital regions, but more marked in the hemisphere contralateral to the visual field deficit (**Figure 4**). Likewise, reduction in white matter integrity, which was widespread, was lateralized to the hemisphere originating the hemianopia but in the occipital lobes only.

## Positron Emission Tomography with Fluorodeoxyglucose (FDG-PET)

Functional imaging is used as evidence of neurodegeneration in PCA, being particularly useful when MRI is considered normal. When examined with FDG-PET, patients with PCA show hypometabolism that is more marked in the occipitoparietal regions, sometimes with involvement of the frontal eye fields, but with relative sparing of the mesial regions of the frontal and temporal lobes (55–57). Directly compared with amnestic AD, PCA patients present more severe occipitoparietal and/or occipital hypometabolism in the right hemisphere (47, 55, 57, 58).

Positron emission tomography with fluorodeoxyglucose has also been studied in the differential diagnosis between PCA and LBD, with variable results (57, 59). A common problem is that, in line with overlapping posterior presentations, occipital hypometabolism may occur in both conditions (7, 60). In a comparative study, patients with PCA showed greater hypometabolism in the right temporooccipital cortex, while LBD was distinguished by hypometabolism predominating in the left occipital cortex (57). In another study (59), however, rather than lateralization, it was the degree of asymmetry and anterior extension that best distinguished them: PCA and LBD were

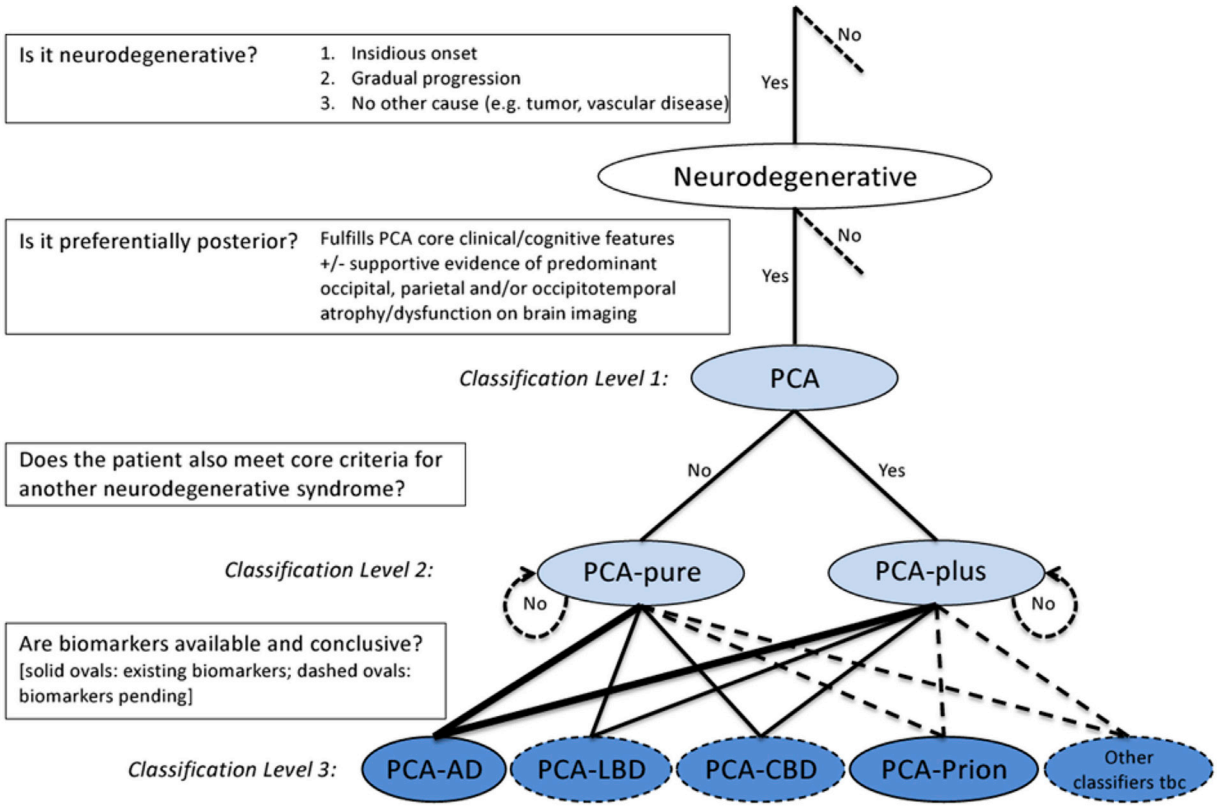


**FIGURE 2** | Diagnostic process and PCA classification. Key diagnostic questions at each level are shown in boxes. Syndrome-level descriptions (classification levels 1 and 2) are lightly shaded and disease-level descriptions (classification level 3) are darkly shaded. Among the disease-level classifications, PCA-AD and PCA-prion (solid ovals) are distinguished from PCA-LBD and PCA-CBD (dashed ovals) owing to the current availability of *in vivo* pathophysiological biomarkers. Other disease-level classifications may be appropriate (e.g., a patient with PCA plus visual hallucinations may have LBD-variant of AD) or anticipated (e.g., PCA attributable to GRN mutations). The thickness of lines connecting classification levels 2 and 3 is intended to reflect the status of AD as the most common cause of PCA. Abbreviations: AD, Alzheimer's disease; CBD, corticobasal degeneration; LBD, Lewy body disease; PCA, posterior cortical atrophy; tbc, to be confirmed. Reproduced from Crutch et al. (52), available under the terms of Creative Commons Attribution License (CC BY 4.0).

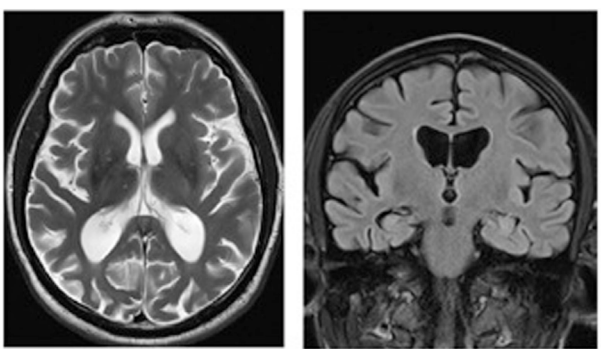

**FIGURE 3** | Magnetic resonance images (axial T2, coronal FLAIR) of a patient with posterior cortical atrophy demonstrating marked regional atrophy in the occipitotemporal regions and relative preservation of the hippocampi. Patient under the care of GTP; published with patient's authorization.

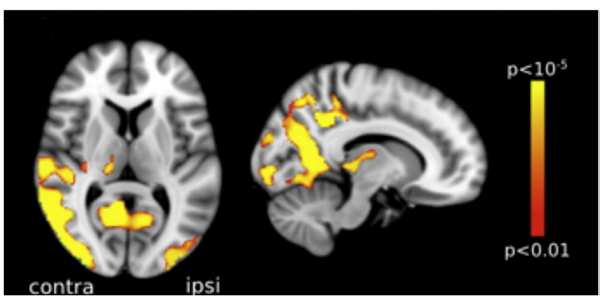


**FIGURE 4** | Voxel-based morphometry analysis comparing a group of posterior cortical atrophy (PCA) patients and age-matched healthy controls. Areas with most significant atrophy (highlighted in red-yellow) in PCA patients included the lateral and anterior occipital cortex, with some loss also noted in the parietal lobe, more marked in the hemisphere contralateral to the visual field defect, here represented on the left. Reproduced from Millington et al. (38), available under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

both associated with bilateral occipitoparietal hypometabolism, but a higher degree of asymmetry favored PCA, while extension to orbitofrontal and anterior temporal regions was suggestive of LBD. The relative preservation of the posterior cingulate cortex compared with the precuneus and cuneus—a sign that has shown to distinguish LBD from typical AD (61, 62)—was not specific to LBD when compared with PCA (59).

### PET Imaging of Microglia

This novel imaging modality is based on the involvement of microglia activation in the pathogenesis of neurodegenerative diseases. The PET tracer $^{11}$C-PBR28 binds to the translocator protein-18 kDa, which is overexpressed in activated microglia; therefore, its regional distribution can be interpreted as an effect of local degeneration (63). In $^{11}$C-PBR28-PET, patients with PCA showed greater binding in occipital, posterior parietal, and temporal regions compared to controls and were distinguished from amnestic AD patients by higher binding in the occipital cortex bilaterally (64). Despite these encouraging results, TPSO PET imaging still has some limitations, including the existence of non-binders (65).

### Dopamine Transporter Imaging

Albeit not part of PCA routine assessment, this test may be very helpful when the clinical diagnosis of LBD is in the differential, as well as in patients with established PCA who progress with LBD features, e.g., parkinsonism and visual hallucinations. The finding of low dopamine transporter concentration in basal ganglia, measured with PET or single photon emission tomography (SPECT), supports the diagnosis of LBD (60).

## ELECTROPHYSIOLOGY STUDIES

The study of event-related potentials (ERPs) involves the quantification of eletroencephalographic changes caused by cognitive, motor, or sensory stimulation. The responses are stereotyped and time-locked with stimulus, permitting analysis of the various stages of the neural process. The best recognized finding of ERP in AD patients is an abnormal P3b (also known as P-300), a higher cortical response associated with an update in working memory (66). Potentials associated with earlier visual processing, such as P1 and N1, may also be abnormal (67). The latter is ascribed to dysfunction of the visual association cortex (67), which is commonly involved by AD pathology (68). Interestingly, in non-symptomatic PSEN1 carriers (preclinical AD) tested with a visual recognition memory task, ERP were reduced in frontal and increased in occipital regions, suggesting that posterior cortical decline in AD is preceded by increased reliance on these regions (69). There is no group study of the visually evoked potential (VEP) in PCA, but single-case reports show that they may be normal (70) or delayed (40). This is likely explained by variable disease stages and clinical presentations. Indeed, Mares et al. (71) studied a PCA patient with pure alexia, showing bilaterally absent N170—an ERP component associated with word reading and activation of the visual word form area—and concomitantly abnormal P1 (initially delayed in the left hemisphere only, later bilaterally), indicating that the early visual cortical dysfunction may have contributed to the reading disorder. Since visual ERPs depend on the integrity of the visual system, the finding of abnormal VEPs may favor the diagnosis of PCA in the appropriate clinical context, but there is no evidence to support their use in the differential diagnosis with amnestic AD.

## AD BIOMARKERS

### Positron Emission Tomography with an Amyloid-binding Tracer (PET-Amyloid)

Amyloid imaging has a role in the diagnosis of AD, more so in presenile patients—when greater deposition of β-amyloid is not expected—but has no use in distinguishing PCA from amnestic AD, as the same global pattern of deposition is seen (17, 72). This is no surprise, since in pathological studies of PCA, the anterior-posterior gradient refers to the disparate concentration of tau-derived neurofibrillary tangles in posterior regions, while the distribution of β-amyloid is diffuse (7).

### Cerebrospinal Fluid (CSF) Analysis with Measurement of AD Pathology Biomarkers

Cerebrospinal fluid examination is not required for the diagnosis of PCA, but this test is usually recommended in presenile dementia to exclude treatable causes. Furthermore, in the current diagnostic approach to AD, CSF biomarkers have a positive predictive value when they show low concentrations of amyloid β, increased total tau, and increased phospho-tau (10). As with the PET-amyloid, the application of these tests to PCA patients did not help to distinguish them from amnestic AD (35), although they have a place in the differential diagnosis of AD and alternative underlying pathologies.

### Tau-PET

Several tau-specific tracers for PET, including $^{18}$F-AV-1451 (Flortaucipir) were recently made available for clinical assessment of various tauopathies (73). Although their affinity for specific tau deposits is still to be established, the application of tau-PET to the diagnosis of AD is promising. Because $^{18}$F-AV-1451 binds to hyperphosphorylated paired helical filament tau and neurofibrillary tangles (73), and tau pathology—but not amyloid—is at disproportionate higher concentration in posterior brain regions in PCA (5, 7), the tau-PET has the additional potential to distinguish PCA from other AD variants. A pattern with localized elevation of $^{18}$F-AV-1451 to posterior regions has indeed been shown to strongly correlate with PCA (74, 75), distinguishing it from amnestic AD (76). Moreover, regional tau-binding mirrors regional patterns of both hypometabolism (74, 75) and atrophy (75) across AD major phenotypes, PCA included. For this reason, it is possible that this test be included as an *in vivo* imaging biomarker of AD in future (75).

### Serum Biomarkers of AD

Decrement of peripheral β-amyloid does occur in AD, but later than CSF levels, so that an important effect is observed at the stage of dementia only (77). Plasma levels of Aβ have not been studied in PCA. Markers of neuronal injury, the tau, and neurofilament light (NFL) proteins are elevated in the sera of AD patients, but levels significantly overlap with those of controls and individuals with mild cognitive impairment, although NFL may be more accurate (78). These difficulties have prevented the recognition of a serum signature of AD. Plasma NFL levels further correlate with longitudinal measures of cognition and

atrophy in AD and have been suggested as a tool for screening patients at risk of cognitive decline (78); however, NFL is not specific for AD, thus cannot help in the differential diagnosis with other neurodegenerative conditions.

## PCA AS A PHENOTYPE WITHIN THE SPECTRUM OF AD CLINICAL VARIABILITY

The predominance of right hemisphere deficits in PCA patients observed in metabolic (47, 55, 57, 58, 79), as well as structural (20, 54, 80) imaging is intriguing. Some of the syndrome's most characteristic deficits correlate with either bilateral (e.g., simultanagnosia) or dominant hemisphere (e.g., Gerstmann syndrome) dysfunction (18). Besides, a selective vulnerability of the right hemisphere cannot be easily hypothesized on a pathological basis. The explanation for such disparity may instead lie with the syndrome definition. Failure in visual object and space perception, which underlies the concept of PCA as a higher order visual disorder, is indeed associated with bilateral occipital and right parietal damage (74, 81). A left hemisphere dysfunction would manifest with predominant language deficits; accordingly, relatively focal left parietal atrophy/hypometabolism is a topographical marker of logopenic primary progressive aphasia (lvPPA) (82), the "language" variant of AD (10). In line with syndrome continuity, when patients with PCA develop language problems, these are usually dominated by word retrieval deficits, a clinical overlap with lvPPA (12).

In clinic, the differentiation between PCA and amnestic AD is more often a challenge, for the latter is common and can also present with visuospatial deficits. The basis for these common deficits is a shared neuroanatomic substrate that critically involves the parietal lobes. This has been identified as the "default model network" (DMN), after the observation that it activates during non-focused rest, which encompasses structures such as the medial-temporal lobe, precuneus, posterior cingulate, and temporoparietal junction (83), with a central role suggested to the precuneus (84). The DMN is commonly affected across AD variants, however at disproportionate, syndrome specific regional severities (11, 85). In typical AD, pathology starts in the entorhinal cortex, then stereotypically spreads to limbic regions then to isocortex, including parietal association cortex (68, 86). In PCA, the higher pathology burden is found in the primary visual and visual association cortices, with the posterior parietal regions being commonly involved as well (5). Likely reflecting pathology, at metabolic imaging, the highest degree of overlap between AD variants localizes to the dorsal DMN (85). From a dynamic perspective, the parietal lobe may thus be seen as a hub where PCA and AD meet, in diverging directions, within a common network of progression. Accordingly, patients with PCA show decreased functional connectivity in the visual network, in various regions—including the precuneus—within the DMN, as well as more anterior structures (87). In addition, white matter loss is more diffuse in PCA than would be expected from its relatively focal posterior presentation (38, 87), likely anticipating more anterior deficits. A consequence of these converging patterns of degeneration between PCA and typical AD is that the diagnosis of patients presenting with focal parietal deficits may be challenging. For instance, in a patient with a relative isolated visuoconstructive disorder and right parietal hypometabolism, the differential diagnosis between AD and PCA may not be accurate until further memory or visual deficits develop, and/or hypometabolism extends to more temporal or occipital regions, respectively, although the preservation of memory and the lateralized right presentation could increase the odds for PCA.

## GENETICS

Posterior cortical atrophy is predominantly a sporadic condition. However, the PCA phenotype has rarely been described in association with genetic mutations known to be implicated in familial AD, [*PSEN1* (88, 89) and *PSEN2* (90)], mutations associated with frontotemporal dementia [*MAPT* (91) and *GRN* genes (8)], as well as in the gene of the prion protein (*PRNP*) (92).

In recent years, there has been an effort to understand the factors driving phenotypic variation in AD. Previous investigations of the commonest genetic risk factor to late onset AD, the allele ε4 of the *APOE* yielded conflicting results in PCA, with some studies suggesting that variation in this gene confers an increased risk to the visual variant of AD (54, 93), while no association was found by others (13, 94). A recently published consortium study, which included the largest number of PCA patients to date, reported a robust association between variation in/near *APOE*/*TOMM40* and risk for PCA, but with a smaller effect than that for amnestic AD (95).

Variants of *TREM2* and *PSEN2* that modify the risk for AD have also been reported in PCA (93), but there is no evidence for any particular effect in PCA as compared to amnestic AD.

## TREATMENT

No study is available reporting the effectiveness of acetylcholinesterase inhibitors in this condition. Nonetheless, given the strong pathological association with AD, most clinicians dealing with PCA patients find it appropriate to offer them a trial of these drugs. Likewise, memantine is sometimes tried in individual cases.

A considerable part of the management of PCA consists of assisting patients in taking decisions about their occupational and daily lives, considering that, albeit slowly, vision and cognition will continue to deteriorate. Among lifestyle changes stopping driving should be recommended. One of us (GTP) considers the condition to be appropriate grounds for registration as "severely sight impaired" which in the UK is equivalent to being registered as "legally blind" despite normal visual acuity in the early stages.

## CONCLUSION

Posterior cortical atrophy, a clinico-radiological syndrome that in most cases represents a focal form of AD, is unique among the known dementing conditions for causing a highly disabling visual disorder with preserved cognitive status in the early stages.

The course of PCA due to AD is stereotyped with virtually all patients later developing memory loss and progressing into full dementia. The recognition of PCA as an atypical variant of AD and the availability of accurate AD biomarkers has made PCA a condition where a diagnosis of definitive AD can be given *in vivo*. When non-AD pathologies are the cause of PCA, clues for the alternative pathology are often found in clinical features as well as imaging.

Despite the progress in the understanding of the neural basis of PCA in recent years, patients still frequently experience a painful delay in diagnosis, mainly because it is not appreciated that their symptoms are associated with brain dysfunction by optometrists and ophthalmologists who are consulted. The need of increasing awareness among clinicians cannot be overestimated, and this should involve not only neurologists, but general practitioners, optometrists, and ophthalmologists.

For all who see patients with visual symptoms we would emphasize the following. First, in the anamnesis, take note of visual symptoms that have an emphasis on spatial disorientation. Second, in the basic clinical assessment, such features as unexplained difficulty with Ishihara plates, variable homonymous defects on perimetry and a tendency to omit letters on the acuity chart should raise suspicion of the disorder.

## AUTHOR CONTRIBUTIONS

GP conceived project and revised early drafts of the article. HB, RM, and MJ-G revised early drafts of the article. MNMdS generated first draft of article.

## FUNDING

MNMdS was supported by a scholarship from the Brazilian Conselho Nacional de Pesquisa (CNPq). RM held a Medical Research Council Studentship and a Foulkes Fellowship. HB is a Royal Society University Research Fellow.

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