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Cholinesterase activity in the eye?

Sandra C Durán-Cristiano[1]

Primary Eye Care Research Group, Health and Sciences Faculty, Optometry Program, Universidad de la Salle. Bogotá, Colombia[1*]

Abstract

The visual system is regulated by the nervous through neurotransmitters, which play an important role in visual and ocular functions. One of those neurotransmitters is acetylcholine, a key molecule that plays a diversity of biological functions. On the other hand, acetylcholinesterase, the enzyme responsible for the hydrolysis of acetylcholine, is implicated in cholinergic function. However, several studies showed that in addition to their enzymatic functions, Acetylcholinesterase exerts non-catalytic functions. In recent years, the importance of evaluating all possible functions of acetylcholine-acetylcholinesterase has been evidenced. Nevertheless, there is evidence that suggests cholinesterase activity in the eye can regulate some biological events both in structures of the anterior and posterior segment of the eye and therefore in the visual information that is processed in the visual cortex. Hence, the evaluation of cholinesterase activity could be a possible marker of alterations in cholinergic activity not only in ocular disease but also in systemic diseases.

Keywords: cholinesterase, acetylcholine, visual function, ocular surface, retina.

[1] Corresponding author
sduran@unisalle.edu.co.

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1. Introduction

The visual system is regulated by a variety of neurotransmitters among them glutamate, ácido gamma aminobutírico (GABA), adrenaline, and acetylcholine that contributed to the spread of nerve impulses in the different types of eye cells, and visual pathways, helping to control the autonomic motor and sensitive response [1–3]. Acetylcholine is a neurotransmitter (NT), that influences various biological processes as well as cell signaling, proliferation, and cellular differentiation, whereby the visual system and specifically the eye as a sensorial organ requires those NT [4,5].

Several studies suggest the role of acetylcholine (Ach), as well as the molecules that intervene in its biogenesis and degradation, in the cortical response and cognitive functions as memory, locomotion, and perceptual learning [5]. The cholinergic signal modulates the synaptic transmission process in different groups of neurons and in the motor end-plate, for which, changes in the cholinergic activity would induce structural and anatomical changes of the cellular components [6].

Although many studies evaluate cholinergic activity at the visual level through a functional test such as measurement of contrast sensitivity, pupil reflex, and tear secretion, few studies demonstrate the role of acetylcholinesterase (AChE) in the eye. Therefore, questions arise among them: Is it possible to evaluate the AChE in tear? What other functions could acetylcholinesterase play in the visual system? ¿Could AChE measurement serves as an indicator of neuronal condition? Therefore, this review will focus on the role of AChE in the visual system and eye (Figure 1), presenting its participation in different ocular tissues and its relevance in the diagnosis of ocular and neuronal diseases.

2. Cholinergic System

Acetylcholine is synthesized from acetyl Coenzyme A and choline mediated by choline acetyltransferase (ChAT), while acetylcholinesterase (AChE) is responsible for its degradation. Both ChAT and AChE are present in the site where Ach is required, such as the muscle, neurons, etc. [7,8]; also, for acetylcholine to send the signal from one cell to another. In addition to its release, it is necessary that acetylcholine bind to specific receptors with high affinities such as muscarinic and nicotinic. Although both receptors present a great affinity for Ach, structural differences arise, being the nicotinic receptors or also called ionotropic transmembrane ion channels, while the muscarinic or metabotropic receptors are G protein-coupled receptors [9,10].

Once Ach is bound with its receptors, it exerts an effector function in the cell and sends the signal to the other cells in a paracrine or autocrine manner [11]. For this, it requires the

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cholinergic activity to enter in a resting state and then reactivated again, for which the role of acetylcholinesterase is elementary for the degradation and to prevent the accumulation of Ach which could cause desensitization or over-stimulation in the receptors and thus affect biological events that depend on its activity [10,12]. However, it is noteworthy that some studies suggest the role of AChE not only for its catalytic activity but also for not-catalytic functions [13], which makes the cholinergic system not only regulate synaptic transmission response but can modulate other functions neuronal and muscular, as we will see later.

The visual system is regulated by the nervous system and therefore, changes in the expression of certain neurotransmitter could affect vision [14] for the mammals include the human, the visually perceive requires not only optimal conditions in the media refractive of the eye and an adequate retinal response but require a visual pathway without any alteration so that the visual cortical areas that interpret the visual information. For this reason, authors such as Disney et al, have demonstrated in an animal model, the presence of nicotinic and muscarinic receptors in the primary visual cortex (V1), as well as the presence of cholinergic activity and cholinesterase in the lateral geniculate nucleus [15]. Thus, visual functions such as contrast sensitivity, color vision stereopsis, among others, could be modulated by the cholinergic system. Thus, as Han et al, experimentally demonstrated, the active visual activity leads to elevated expression of nicotinic receptors in visual cortical areas and changes in pupillary size may be related to muscarinic activity in the iris and pupillary sphincter [16].

The visual system that includes the eye and the retina-genicular-cortical pathway requires the presence of the cholinergic activity and its action varies depending on the different nicotinic and muscarinic receptors present in each structure so that some changes in the cholinergic activity either in the synthesis or biogenesis of Ach as well as the degradation and binding of its receptors would result in a change in the answer in the visual function. Despite finding a large number of publications on the influence of the cholinergic system on visual function, very few articles report the activity of acetylcholinesterase (AChE) in the visual system, therefore this review will focus on the role of AChE in the visual system, presenting its participation in different ocular tissues and its relevance in the diagnosis of ocular and neuronal diseases. Throughout the document, an attempt will be made to explain some concepts that could solve some concerns about the enzyme responsible for degrading Ach: AChE

2.1 Acetylcholinesterase

It is an enzyme with hydrolase esterase function for Ach, being hydrolase in the ester type bond, structurally it appears as a homomeric assembly with three catalytic subunits H, T, and S, being different in the C-terminal region. The soluble form can found in the cell membrane and extracytoplasmatic regions [17,18]. Human AChE is encoded on chromosome 7q22 and three subunits of AChE are produced through alternative splicing, which differs

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in the site of cell localization, taking into account that the places where this enzyme is expressed are in cholinergic neurons, attached to the erythrocyte membrane and in the membrane of postsynaptic neurons including the neuromuscular junctions [17].

The catalytic site of AChE allows the specific binding to Ach in an irreversible way, achieving the transformation into acetate and choline after hydrolysis, the latter can be reuptained by a choline transporter within the terminal nerve fibers [19].

The non-enzymatic activity of AChE

AChE has been attributed important functions from neurodevelopmental to the modulation of various higher cognitive functions; from developmental, AChE acts as a nerve growth factor, stimulating the process of neurogenesis and synaptogenesis [13,20,21]. Indeed, it plays an important role in the development of neuroectoderm structures such as the ciliary body, retina, and optic nerve. In some in vitro models, the presence of AChE in the plasma region has been suggested where various non-catalytic motifs interact with laminin, collagen IV and intervene in cell adhesion, migration, and neuronal growth [22], for which different ocular tissues of the neural origin may include such functions. Thus, Slotkin et al, observed in an in vivo model, that AChE intervenes in the formation of neurites and stability of neural networks[13], suggesting that events that induce changes in the expression of AChE as exposure to pesticides, including Organophosphates and carbamates, could induce changes in the formation and growth of neuritis and thus the development of neurocognitive functions such as learning and memory [23].

3. AChE activity in the posterior segment of the eye:

Retina and optic nerve:

The characteristics of both the retina and optic nerve make them structures like windows of the nervous system (NS); in fact, some authors describe these structures as prolongations of the NS [24]. Consequently, the retinal neuronal activity includes the participation not only of NTs such as glutamate and GABA [25–27]. Besides, several studies show that retinal functionality requires autonomic activity. Indeed, some researchers have reported the presence of muscarinic receptors in cells as photoreceptors and amacrine, as well as the presence of nicotinic receptors in retinal glial cells (Müller Cell), bipolar and retinal ganglion cells [3,28,29].

Although some studies focus on the evaluation of the parasympathetic cholinergic activity by determining the presence of Ach receptors [25,26], other studies suggest instead, the evaluation of cholinesterase activity in retinal tissue as part of the parasympathetic evaluation. For instance, Nichols et al, demonstrated high esterase activity in rabbit retina, in different cell types of the internal layers, such as the amacrine and ganglion retinal cells

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[30], which suggest that in intraretinal areas, nerve conduction requires cholinergic activity [31]. In addition, Glow & Rose suggest that changes in cholinesterase activity may be related to efferent pupillary response defects that may occur to retinal and optic nerve injury [32,33].

The combination of clinical, histological, and molecular studies has allowed us to better characterize the cholinergic activity in the different layers of the retina, finding a relationship between retinal functional changes with parasympathetic dysfunctions, the foregoing by evaluating retinal cholinesterase activity [30]. However, despite describing the role of cholinesterase in retinal cholinergic activity due to its catalytic activity on acetylcholine, other studies describe non-catalytic functions in the retina, as reported by Blasina et al, and Appleyard et al, who demonstrated that AChE independent of catalytic activity modulates retinal development and differentiation apart from protease and/or peptidase activity expressed in neurites during the neuronal development process [34,35]. Therefore, in recent years, studies such as that of Almasieh et al, reveal that the inhibition of AChE favors the vasoprotection of the retina and increases ocular blood flow [36], events that are crucial in glaucomatous disease and other vascular retinal disorders.

4. AChE activity in the Anterior segment of the eye:

Some research suggests the role of parasympathetic autonomic activity in the anterior segment to the presence of muscarinic receptors in the iris sphincter muscle, ciliary muscle, ciliary processes, trabecular meshwork, and lacrimal gland [37]. However, other studies demonstrate that ocular surface structures may be cholinergically regulated mediated by AChE activation as described below.

Uveal tract:

Parasympathomimetic activity regulates a large part of the iris functions, allowing to induce modifications in the pupillary response, and therefore in the amount of light stimulus that leads to the retina. Studies in rabbits and cats have found that the cholinesterase activity in the iris structure is increased in visual activities [38]. Interestingly, Hayakawa et al, found in a murine model that exposure to organophosphates whose objective is to inhibit cholinesterase, decreases AChE activity in the Edinger Westphal nucleus, iris, and pretectal nucleus, aspects that correlated with the pupillary miotic response [39].

On the other hand, a high cholinesterase activity has been reported in pars plana and in the pigment epithelium of the ciliary body [40], this has been demonstrated by analysis of the ciliary muscle before and after ciliary ganglionectomy, where cholinesterase activity is affected later to the surgical act [41]. The active presence of cholinesterase, both in the ciliary muscle and ciliary body could explain the association between uveitic processes associated with autonomic dysregulation [42] and in some cases with changes in the accommodative functions in individuals exposed to organophosphates, whose cholinesterase is decreased [43].

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Regarding the ciliary body, some differences between the results may be due to the animal model and the biochemical techniques used for the evaluation of AChE activity, for example, Gabelt et al, found in experiments with macaques a higher AChE activity that could be related to a greater parasympathetic demand on the ciliary muscle [44]. Another study found that inhibition of AChE activity reduces blood flow in the anterior uvea and induces pupillary miosis [45]. This suggests that cholinesterase activity plays a role in functions in the uveal tract to maintain intraocular homeostasis.

Anterior Chamber

Some studies suggest the presence of AChE in the anterior chamber, this being the one that contributes greatly to the nutrition and metabolism of the iris, ciliary and crystalline bodies. Previous studies in cats have described the activity of AChE from studies with paracentesis, where it is possible to obtain aqueous humor analysis [46], this could explain to some extent, why autonomic dysregulation plays an important role in glaucoma diseases and how AChE inhibitors could be included in glaucoma pharmacotherapeutics [47].

Consequently, for several decades it has been mentioned that from paracentesis it is possible to extract aqueous humor and evaluate cholinesterase activity. However, Appleyard et al. found that cholinesterase activity in ocular fluids is attributed more to true cholinesterase than to butyrylcholinesterase using Ellman´s method. Curiously, higher cholinesterase activity was observed in the vitreous humor and posterior cavities, so the authors suggest that the origin of this activity can occur from retinal tissue [35]. Despite, underestimating BuChE activity for years as a cholinergic indicator and recent studies in fluids such as saliva, and serum, attribute its importance in the measurement of cholinergic activity [48,49]. Therefore, the fact that there is cholinesterase activity of BuChE could be associated with the parasympathetic state.

Cornea:

The cornea is an avascular structure, which is in communication with the tear film and aqueous humor, this structure presents a sensitive response accompanied by somatosensory activity due to the presence of thermoreceptors, polymodal receptors, and mechanoreceptors, responding to sensations such as pain, temperature and chemical changes [50]. The reception of the visual stimulus generates a series of cellular and molecular events in the cornea, of which certain NTs such as substance P and acetylcholine [51]. In an animal model, cholinesterase activity has been described in chicken corneal extracts, as well as the AChE reaction in corneal layers such as corneal epithelium and stroma in rats and rabbits [52,53], which could suggest a regulatory element of corneal sensoriality. Lasys et

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al. found cholinesterase activity in the corneal nerve plexus [54] and a study in corneal epithelial cell culture showed that the epithelium expresses mAchR, and AChE, which contribute to cell migration and survival [55].

Consequently, some studies have evaluated the activity of both the specific cholinesterase and pseudocholinesterase in corneal nerve fibers in different species of animals, and changes in the corneal sensory response, could affect the functionality of AChE. Thus, raising the possibility of find changes in AchE activity in corneal alterations such as herpetic keratitis, diabetic keratopathy, dry eye, corneal surgery, these changes are related to changes in the morphology of corneal nerve fibers using in vivo microscopy [56]. Interestingly, a study suggests, the participation of the cholinergic system in the modulation of the immune response and corneal repair, taking into account the presence of Ach and its binding with receptors present in different types of immune cells such as macrophages and T lymphocytes [57] and therefore the consequent cholinesterase activity to allow the action of Ach.

Lacrimal Gland and conjunctiva

Although the expression of acetylcholine and choline acetyltransferase may be greater than cholinesterase, both in conjunctival and corneal epithelium, the role of their activity is unknown. Some reports, such as Wilson & McKean, associate the cholinergic activity in these epithelia in cellular repair processes, where the activity increases together with glycogen and oxidative enzymes [58]. On the other hand, the use of pesticides such as carbamates seeks cholinesterase inhibitors, can affect the cholinergic terminals in conjunctiva tissue [59] and this can be associated with the over-stimulation that occur in the muscarinic receptors located in conjunctival tissue, which generates a change in the effector response, resulting in the presence of symptoms such as eye irritation, burning, and redness. This may be associated with the activation of intracellular signals, such as the MAPK pathway, which can occur when faced with excessive cholinergic stimulation [60].

In the case of Globet cells of the conjunctiva, in the pathophysiological process such as dry eye and allergic conjunctivitis, it can increase mucin production through the interaction of vasoactive intestinal peptide (VIP) and acetylcholine with muscarinic receptors M2 and M3, which can generate the activation of intracellular signaling cascades that induce the secretion of inflammatory mediators [61,62]. In this sense, it could be thought that cholinesterase is part of the regulatory elements to know when the binding of receptors with Ach must continue and when it must exert its hydrolysis function so that Ach is reuptained and the function is changed.

In the lacrimal gland, cholinergic activity can be mediated by the interaction between ACh and M3 receptors, located in secretory globular acinal cells and in myoepithelial cells to stimulate tear secretion [63]. Nevertheless, this secretion process must be counteracted with

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elimination mainly via the nasolacrimal duct. Therefore, excessive lacrimation generates an imbalance between tear secretion and excretion process, something typical in cholinergic syndrome associated with the use of pesticides such as cholinesterase inhibitors [64]. The above suggests that cholinesterase activity is necessary to maintain tear homeostasis.

5. Is it possible to find AChE activity in tear?

Tear fluid, in recent decades, has had great clinical importance, in the application of molecular and cellular biology techniques. From this fluid, its small volume has not impeded for it to search for biomolecules such as proteins, mRNA, enzymes, and even for small non-coding RNAs, that contribute to gene expression and are involved in the development of various ocular disorders such as dry eye, keratoconus, glaucoma, etc. [65–68].

Therefore, it is possible to think that from the tear fluid, information can be obtained on biomolecules that intervene not only in ocular homeostasis but also affect systemic diseases such as metabolic and neurological conditions. For this reason, ocular fluids could be a source of biomarkers [69–72]. This can be applied to the case of AChE, although some studies suggest that the activity of the enzyme occurs in different ocular structures that have parasympathetic activity, other studies, provide information on the cholinergic influence on tear secretion, therefore it is possible to think that both enzyme activity and its expression could eventually participate in the regulation of various functions in the anterior segment of the eye.

Several studies show that the measurement of AChE is possible in body fluid such as blood, serum, urine, saliva, and cerebrospinal fluid, so it could be speculated that through the tear fluid the activity can be evaluated by enzymatic assay of AChE or BuChE [49,73], considering the volumes required for the reaction [74]. For example, Ellman´s method is one of the most used assays to evaluate BuChE, and although some authors describe its activity as not specified, others show that BuChE activity in saliva can provide relevant information on cholinergic alterations such as the area in two particular cases: Alzheimer´s disease and neurotoxicity due to pesticides, where it occurs as a loss of cholinergic neurons, two situations that area of the public health problems worldwide [48,75,76].

Furthermore, Thiphom et al. demonstrated that in farmers exposed to pesticides, the plasma and saliva levels of BuChE are correlated in the two fluids and decreases in the population exposed to pesticides [49], in this sense, the possibility of evaluating the enzymatic activity in tear fluids, is highly interesting due to its low cost and less invasiveness. Therefore, I suggest, research to evaluated non only AChE in saliva but also in tears using a test with greater sensitivity, even though the concentrations of some biomolecules that are evidenced

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in blood, in the tear show a lower concentration, and additional evaluation is correlated with other visual and ocular functions with cholinergic activity.

6. Future Directions

From the applications of molecular biology to health science, it has been possible to estimate several biological events that intervene in ocular diseases of clinical importance, such as Keratoconus, Dry eye, and Glaucoma [67,77,78]. These advances have allowed the application of new technological tools for the diagnosis and monitoring of ocular or systemic alterations from ocular structures [79]. Although to data, the publications on AChE in ocular structure is evident, the presence of the enzymatic activity of AChE in tear is unknown, so it could be a new research door that will not only be applied to visual professionals, but it could be a good source of research from the area of Neuroscience applied to scenarios such as neurotoxicity by pesticides, and Alzheimer´s disease, taking into account that the AChE evaluation in various fluids including "tears" it could be used as a biomarker of the disease and correlate it with visual and ocular functions that require cholinergic regulation.

7. Conclusions

The cholinesterase activity in the eye is very important in visual and structural functions, its necessary for manteinance and regulation in the cholinergic system, from enzymatic activity to not enzymatic. For this reason, its possible to think that in cholinergic disease (upregulation or downregulation), the AChE in eye affects its role and the structures of the anterior and posterior segment of the eye such as cornea, conjunctiva, lacrimal gland, uveal tract and retina. Ultimately, novel researchs suggest evaluate a variety of biomarkers in ocular and systemic disease, therefore, the AChE as part of cholinergic system could be evaluate through of tear film.

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8. References

1. Takeuchi T, Yoshimoto S, Shimada Y, Kochiyama T, Kondo HM. Individual differences in visual motion perception and neurotransmitter concentrations in the human brain. *Philos Trans R Soc B Biol Sci*. 2017;372(1714):20160111. doi:10.1098/rstb.2016.0111

2. Skangiel-Kramska J. Neurotransmitter systems in the visual cortex of the cat: possible involvement in plastic phenomena. *Acta Neurobiol Exp (Wars)*. 1988;48(6):335-370.

3. Nietgen GW, Schmidt J, Hesse L, Hönemann CW, Durieux ME. Muscarinic receptor functioning and distribution in the eye: Molecular basis and implications for clinical diagnosis and therapy. *Eye*. 1999;13(3):285-300. doi:10.1038/eye.1999.78

4. Shimegi S, Kimura A, Sato A, et al. Cholinergic and serotonergic modulation of visual information processing in monkey V1. *J Physiol Paris*. 2016;110(1-2):44-51. doi:10.1016/j.jphysparis.2016.09.001

5. Kang J Il, Huppé-Gourgues F, Vaucher E. Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. *Front Syst Neurosci*. 2014;8:172. doi:10.3389/fnsys.2014.00172

6. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron*. 2012;76(1):116-129. doi:10.1016/j.neuron.2012.08.036

7. Amenta F, Tayebati SK. Pathways of acetylcholine synthesis, transport and release as targets for treatment of adult-onset cognitive dysfunction. *Curr Med Chem*. 2008;15(5):488-498. doi:10.2174/092986708783503203

8. Ofek K, Soreq H. Cholinergic involvement and manipulation approaches in multiple system disorders. *Chem Biol Interact*. 2013;203(1):113-119. doi:10.1016/j.cbi.2012.07.007

9. Jones CK, Byun N, Bubser M. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology*. 2012;37(1):16-42. doi:10.1038/npp.2011.199

10. Ochoa ELM, Chattopadhyay A, McNamee MG. *Desensitization of the Nicotinic Acetylcholine Receptor: Molecular Mechanisms and Effect of Modulators*. Vol 9.; 1989. doi:10.1007/BF00713026

11. Proskocil BJ, Sekhon HS, Jia Y, et al. Acetylcholine is an autocrine or paracrine hormone synthesized and secreted by airway bronchial epithelial cells. *Endocrinology*. 2004;145(5):2498-2506. doi:10.1210/en.2003-1728

12. Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors:

Shaping cholinergic signaling. *Trends Neurosci*. 2005;28(7):371-378. doi:10.1016/j.tins.2005.04.009

13. Slotkin TA, Ryde IT, Wrench N, Card JA, Seidler FJ. Nonenzymatic role of acetylcholinesterase in neuritic sprouting: regional changes in acetylcholinesterase and choline acetyltransferase after neonatal 6-hydroxydopamine lesions. *Neurotoxicol Teratol*. 2009;31(3):183-186. doi:10.1016/j.ntt.2008.12.007

14. Gollisch T. Throwing a glance at the neural code: rapid information transmission in the visual system. *HFSP J*. 2009;3(1):36-46. doi:10.2976/1.3027089

15. Disney AA, Reynolds JH. Expression of m1-type muscarinic acetylcholine receptors by parvalbumin-immunoreactive neurons in the primary visual cortex: a comparative study of rat, guinea pig, ferret, macaque, and human. *J Comp Neurol*. 2014;522(5):986-1003. doi:10.1002/cne.23456

16. Han ZY, Le Novère N, Zoli M, Hill JAJ, Champtiaux N, Changeux JP. Localization of nAChR subunit mRNAs in the brain of Macaca mulatta. *Eur J Neurosci*. 2000;12(10):3664-3674. doi:10.1046/j.1460-9568.2000.00262.x

17. Grisaru D, Sternfeld M, Eldor A, Glick D, Soreq H. Structural roles of acetylcholinesterase variants in biology and pathology. *Eur J Biochem*. 1999;264(3):672-686. doi:10.1046/j.1432-1327.1999.00693.x

18. UNIPROT. Acetylcholinesterase Human. https://www.uniprot.org/uniprot/P22303

19. Johnson G, Moore SW. Cholinesterases modulate cell adhesion in human neuroblastoma cells in vitro. *Int J Dev Neurosci*. 2000;18(8):781-790. doi:10.1016/S0736-5748(00)00049-6

20. Thullbery MD, Cox HD, Schule T, Thompson CM, George KM. Differential localization of acetylcholinesterase in neuronal and non-neuronal cells. *J Cell Biochem*. 2005;96(3):599-610. doi:10.1002/jcb.20530

21. Day T, Greenfield SA. A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurones in vitro: molecular mechanisms. *Neuroscience*. 2002;111(3):649-656. doi:10.1016/s0306-4522(02)00031-3

22. Johnson G, Moore SW. Identification of a structural site on acetylcholinesterase that promotes neurite outgrowth and binds laminin-1 and collagen IV. *Biochem Biophys Res Commun*. 2004;319(2):448-455. doi:10.1016/j.bbrc.2004.05.018

23. Brimijoin S, Koenigsberger C. Cholinesterases in neural development: new findings and toxicologic implications. *Environ Health Perspect*. 1999;107 Suppl(Suppl 1):59-64. doi:10.1289/ehp.99107s159

24. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. *Nat Rev Neurol*. 2013;9(1):44-53. doi:10.1038/nrneurol.2012.227

25. Hutchins JB, Hollyfield JG. Acetylcholine receptors in the human retina. *Invest Ophthalmol Vis Sci*. 1985;26(11):1550-1557.

26. Ford KJ, Feller MB. Assembly and disassembly of a retinal cholinergic network. *Vis Neurosci*. 2012;29(1):61-71. doi:10.1017/S0952523811000216

27. Hoon M, Sinha R, Okawa H, et al. Neurotransmission plays contrasting roles in the maturation of inhibitory synapses on axons and dendrites of retinal bipolar cells. *Proc Natl Acad Sci*. 2015;112(41):12840 LP - 12845. doi:10.1073/pnas.1510483112

28. Strang CE, Renna JM, Amthor FR, Keyser KT. Muscarinic acetylcholine receptor localization and activation effects on ganglion response properties. *Invest Ophthalmol Vis Sci*. 2010;51(5):2778-2789. doi:10.1167/iovs.09-4771

29. Strang CE, Long Y, Gavrikov KE, Amthor FR, Keyser KT. Nicotinic and muscarinic acetylcholine receptors shape ganglion cell response properties. *J Neurophysiol*. 2014;113(1):203-217. doi:10.1152/jn.00405.2014

30. Nichols CW, Koelle GB. Acetylcholinesterase: method for demonstration in amacrine cells of rabbit retina. *Science*. 1967;155(3761):477-478. doi:10.1126/science.155.3761.477

31. Pourcho RG, Osman K. Acetylcholinesterase localization in cat retina: a comparison with choline acetyltransferase. *Exp Eye Res*. 1986;43(4):585-594. doi:10.1016/s0014-4835(86)80025-2

32. Glow PH, Rose SAM. Effects of Light and Dark on the Acetylcholinesterase Activity of the Retina. *Nature*. 1964;202(4930):422-423. doi:10.1038/202422b0

33. Hall CA, Chilcott RP. Eyeing up the Future of the Pupillary Light Reflex in Neurodiagnostics. *Diagnostics (Basel, Switzerland)*. 2018;8(1):19. doi:10.3390/diagnostics8010019

34. Blasina MF, Faria AC, Gardino PF, et al. Evidence for a noncholinergic function of acetylcholinesterase during development of chicken retina as shown by fasciculin. *Cell Tissue Res*. 2000;299(2):173-184. doi:10.1007/s004419900117

35. Appleyard ME, McDonald B, Benjamin L. Presence of a soluble form of acetylcholinesterase in human ocular fluids. *Br J Ophthalmol*. 1991;75(5):276-279. doi:10.1136/bjo.75.5.276

36. Almasieh M, MacIntyre JN, Pouliot M, et al. Acetylcholinesterase Inhibition Promotes Retinal Vasoprotection and Increases Ocular Blood Flow in Experimental Glaucoma. *Invest Ophthalmol Vis Sci*. 2013;54(5):3171-3183. doi:10.1167/iovs.12-11481

37. Sastry B V. Cholinergic systems and multiple cholinergic receptors in ocular tissues. *J Ocul Pharmacol*. 1985;1(2):201-226. doi:10.1089/jop.1985.1.201

38. Wang Y, Zekveld AA, Naylor G, et al. Parasympathetic Nervous System

Dysfunction, as Identified by Pupil Light Reflex, and Its Possible Connection to Hearing Impairment. *PLoS One*. 2016;11(4):e0153566. https://doi.org/10.1371/journal.pone.0153566

39. Hayakawa S, Hiramoto D, Sekiya H. [Inhibition of acetylcholinesterase at pupil-related central nuclei by organophosphorus pesticide (fenthion)--an experimental study]. *Nihon Ganka Gakkai Zasshi*. 1989;93(2):167-173.

40. deROETTH AJ. Cholinesterase activity in ocular tissues and fluids. *Arch Ophthalmol (Chicago, Ill 1929)*. 1950;43(6):1004-1025. doi:10.1001/archopht.1950.00910011021005

41. Erickson-Lamy KA, Johnson CD, True-Gabelt B, Kaufman PL. Ciliary muscle choline acetyltransferase and acetylcholinesterase after ciliary ganglionectomy. *Exp Eye Res*. 1990;51(3):295-299. doi:10.1016/0014-4835(90)90026-q

42. Yuen BG, Tham VM, Browne EN, et al. Association between Smoking and Uveitis: Results from the Pacific Ocular Inflammation Study. *Ophthalmology*. 2015;122(6):1257-1261. doi:10.1016/j.ophtha.2015.02.034

43. Rengstorff RH. Vision and ocular changes following accidental exposure to organophosphates. *J Appl Toxicol*. 1994;14(2):115-118. doi:https://doi.org/10.1002/jat.2550140213

44. Gabelt BT, Kaufman PL, Polansky JR. Ciliary muscle muscarinic binding sites, choline acetyltransferase, and acetylcholinesterase in aging rhesus monkeys. *Invest Ophthalmol Vis Sci*. 1990;31(11):2431-2436.

45. Silveira R, Stjernschantz J. Vascular effects of acetylcholinesterase inhibitors in the rabbit eye: a study with fasciculin and physostigmine. *J Ocul Pharmacol*. 1992;8(2):129-137. doi:10.1089/jop.1992.8.129

46. Cioboata M, Anghelie A, Chiotan C, Liora R, Serban R, Cornăcel C. Benefits of anterior chamber paracentesis in the management of glaucomatous emergencies. *J Med Life*. 2014;7 Spec No.(Spec Iss 2):5-6. https://pubmed.ncbi.nlm.nih.gov/25870663

47. Almasieh M, Zhou Y, Kelly ME, Casanova C, Di Polo A. Structural and functional neuroprotection in glaucoma: role of galantamine-mediated activation of muscarinic acetylcholine receptors. *Cell Death Dis*. 2010;1(2):e27. doi:10.1038/cddis.2009.23

48. Sayer R, Law E, Connelly PJ, Breen KC. Association of a salivary acetylcholinesterase with Alzheimer's disease and response to cholinesterase inhibitors. *Clin Biochem*. 2004;37(2):98-104. doi:10.1016/j.clinbiochem.2003.10.007

49. Thiphom S, Prapamontol T, Chantara S, Mangklabruks A, Suphavilai C. A method for measuring cholinesterase activity in human saliva and its application to farmers and consumers. *Anal Methods*. 2013;5(18):4687-4693. doi:10.1039/C3AY40269J

50. Dieckmann G, Fregni F, Hamrah P. Neurostimulation in dry eye disease—past, present, and future. *Ocul Surf*. 2019;17(1):20-27. doi:10.1016/j.jtos.2018.11.002

51. Tervo T, Tervo K, Eränkö L, Vannas A, Eränkö O, Cuello AC. Substance P immunoreaction and acetylcholinesterase activity in the cornea and Gasserian ganglion. *Ophthalmic Res*. 1983;15(6):280-288. doi:10.1159/000265273

52. PETERSEN RA, LEE K-J, DONN A. Acetylcholinesterase in the Rabbit Cornea. *Arch Ophthalmol*. 1965;73(3):370-377. doi:10.1001/archopht.1965.00970030372016

53. Tervo T. Histochemical demonstration of cholinesterase activity in the cornea of the rat and the effect of various denervations on the corneal nerves. *Histochemistry*. 1976;47(2):133-143. doi:10.1007/BF00492561

54. Lasys V, Stanevicius E, Zamokas G. Evaluation of peculiarities of the acetylcholinesterase-positive nerve plexus and its length in the cornea. *Medicina (Kaunas)*. 2003;39(10):955-959.

55. Chernyavsky AI, Galitovskiy V, Shchepotin IB, Jester J V., Grando SA. The acetylcholine signaling network of corneal epithelium and its role in regulation of random and directional migration of corneal epithelial cells. *Invest Ophthalmol Vis Sci*. 2014;55(10):6921-6933. doi:10.1167/iovs.14-14667

56. Eguchi H, Hiura A, Nakagawa H, Kusaka S, Shimomura Y. Corneal Nerve Fiber Structure, Its Role in Corneal Function, and Its Changes in Corneal Diseases. Pierscionek BK, ed. *Biomed Res Int*. 2017;2017:3242649. doi:10.1155/2017/3242649

57. Xue Y, He J, Xiao C, et al. The mouse autonomic nervous system modulates inflammation and epithelial renewal after corneal abrasion through the activation of distinct local macrophages. *Mucosal Immunol*. 2018;11(5):1496-1511. doi:10.1038/s41385-018-0031-6

58. Wilson WS, McKean CE. Regional distribution of acetylcholine and associated enzymes and their regeneration in corneal epithelium. *Exp Eye Res*. 1986;43(2):235-242. doi:https://doi.org/10.1016/S0014-4835(86)80091-4

59. National Research Council. ANTICHOLINESTERASES. In: *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents: Volume 1 Anticholinesterases and Anticholinergics.* National A. ; 1982. https://www.ncbi.nlm.nih.gov/books/NBK217772/

60. Horikawa Y, Shatos MA, Hodges RR, et al. Activation of mitogen-activated protein kinase by cholinergic agonists and EGF in human compared with rat cultured conjunctival goblet cells. *Invest Ophthalmol Vis Sci*. 2003;44(6):2535-2544. doi:10.1167/iovs.02-1117

61. Ríos JD, Forde K, Diebold Y, Lightman J, Zieske JD, Dartt DA. Development of conjunctival goblet cells and their neuroreceptor subtype expression. *Invest Ophthalmol Vis Sci*. 2000;41(8):2127-2137.

62. Ríos JD, Zoukhri D, Rawe IM, Hodges RR, Zieske JD, Dartt DA. Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion. *Invest Ophthalmol Vis Sci*. 1999;40(6):1102-1111.

63. Mitchelson F. Muscarinic receptor agonists and antagonists: effects on ocular function. *Handb Exp Pharmacol*. 2012;(208):263-298. doi:10.1007/978-3-642-23274-9_12

64. Caro-Gamboa, L. J., Forero-Castro, M., & Dallos-Báez AE. Cholinesterase inhibition as a biomarker for the surveillance of the occupationally exposed population to organophosphate pesticides. *Cienc y Tecnol Agropecu*. 2020;21(3):e1562. doi:https://doi.org/10.21930/rcta.vol21_num3_art:1562

65. Kim YJ, Yeon Y, Lee WJ, et al. Comparison of microRNA expression in tears of normal subjects and Sjögren syndrome patients. *Investig Ophthalmol Vis Sci*. 2019;60(14):4889-4895. doi:10.1167/iovs.19-27062

66. Izzotti A, Ceccaroli C, Longobardi MG, et al. Molecular Damage in Glaucoma: from Anterior to Posterior Eye Segment. The MicroRNA Role. *MicroRNA (Shariqah, United Arab Emirates)*. 2015;4(1):3-17. doi:10.2174/2211536604666150707124640

67. Nishtala K, Pahuja N, Shetty R, Nuijts RMMA, Ghosh A. Tear biomarkers for keratoconus. *Eye Vis*. 2016;3(1):1-7. doi:10.1186/s40662-016-0051-9

68. Jung JH, Ji YW, Hwang HS, et al. Proteomic analysis of human lacrimal and tear fluid in dry eye disease. *Sci Rep*. 2017;7(1):1-11. doi:10.1038/s41598-017-13817-y

69. Cunha JP, Moura-Coelho N, Proença RP, et al. Alzheimer's disease: A review of its visual system neuropathology. Optical coherence tomography—a potential role as a study tool in vivo. *Graefe's Arch Clin Exp Ophthalmol*. 2016;254(11):2079-2092. doi:10.1007/s00417-016-3430-y

70. Uhlhaas PJ, Pantel J, Lanfermann H, et al. Visual Perceptual Organization Deficits in Alzheimer's Dementia. *Dement Geriatr Cogn Disord*. 2008;25(5):465-475. doi:10.1159/000125671

71. Yap TE, Davis BM, Guo L, Normando EM, Cordeiro MF. Annexins in Glaucoma. *Int J Mol Sci*. 2018;19(4). doi:10.3390/ijms19041218

72. Schicht M, Garreis F, Hartjen N, et al. SFTA3 - A novel surfactant protein of the ocular surface and its role in corneal wound healing and tear film surface tension. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-28005-9

73. Darreh-Shori T, Kadir A, Almkvist O, et al. Inhibition of acetylcholinesterase in CSF versus brain assessed by 11C-PMP PET in AD patients treated with galantamine. *Neurobiol Aging*. 2008;29(2):168-184. doi:https://doi.org/10.1016/j.neurobiolaging.2006.09.020

74. Pohanka M, Hrabinova M, Kuca K, Simonato J-P. Assessment of acetylcholinesterase activity using indoxylacetate and comparison with the standard

Ellman's method. *Int J Mol Sci*. 2011;12(4):2631-2640. doi:10.3390/ijms12042631

75. Claus Henn B, McMaster S, Padilla S. Measuring cholinesterase activity in human saliva. *J Toxicol Environ Health A*. 2006;69(19):1805-1818. doi:10.1080/15287390600631458

76. Benitez A, Ramírez-Vargas MA. Cholinesterase as a biomarker to identify cases of pesticide poisoning. *Mex J Med Res ICSA*. 2021;9(17):47-55. doi:10.29057/mjmr.v9i17.5577

77. Soria J, Acera A, Merayo-Lloves J, et al. Tear proteome analysis in ocular surface diseases using label-free LC-MS/MS and multiplexed-microarray biomarker validation. *Sci Rep*. 2017;7(1):1-15. doi:10.1038/s41598-017-17536-2

78. Can Demirdöğen B, Koçan Akçin C, Özge G, Mumcuoğlu T. Evaluation of tear and aqueous humor level, and genetic variants of connective tissue growth factor as biomarkers for early detection of pseudoexfoliation syndrome/glaucoma. *Exp Eye Res*. 2019;189. doi:10.1016/j.exer.2019.107837

79. Duran Cristiano, S. C., & Gómez-Molina, A. Biomarcadores en película lagrimal y su aplicación clínica. *Revista Salud Bosque*, 2020;*10*(1). https://doi.org/10.18270/rsb.v10i1.2787