Archives Italiennes de Biologie, 160: 3-21, 2022. DOI 10.12871/000398292022121

Combined pulses of light and sound in the retina with nutraceuticals may enhance the recovery of foveal holes

ROBERTO PINELLI[1], CATERINA BERTI[1], ELENA SCAFFIDI[1], GLORIA LAZZERI[2], VIOLET VAKUNSETH BUMAH[3], RICCARDO RUFFOLI[2], FRANCESCA BIAGIONI[4], CARLA LETIZIA BUSCETI[4], STEFANO PUGLISI-ALLEGRA[4], FRANCESCO FORNAI[2,4]

[1] SERI, Switzerland Eye Research Institute, Lugano, Switzerland
[2] Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
[3] Department of Chemistry and Biochemistry College of Sciences San Diego State University 5500 Campanile Drive San Diego, CA 92182, USA
[4] IRCCS Neuromed Pozzili (IS), Italy

ABSTRACT

The present manuscript stems from evidence, which indicates that specific wavelength produce an activation of the autophagy pathway in the retina. These effects were recently reported to synergize with the autophagy-inducing properties of specific phytochemicals. The combined administration of photo-modulation and phytochemicals was recently shown to have a strong potential in eliciting the recovery in the course of retinal degeneration and it was suggested as a non-invasive approach named "Lugano protocol" to treat age-related macular degeneration (AMD). Recent translational findings indicate that the protective role of autophagy may extend also to acute neuronal injuries including traumatic neuronal damage. At the same time, very recent investigations indicate that autophagy activation and retinal anatomical recovery may benefit from sound exposure. Therefore, in the present study, the anatomical rescue of a traumatic neuronal loss at macular level was investigated in a patient with idiopathic macular hole by using a combined approach of physical and chemical non-invasive treatments. In detail, light exposure was administered in combination with sound pulses to the affected retina. This treatment was supplemented by phytochemicals known to act as autophagy inducers, which were administered orally for 6 months. This combined administration of light and sound with nutraceuticals reported here as Advanced Lugano's Protocol (ALP) produced a remarkable effect in the anatomical architecture of the retina affected by the macular hole. The anatomical recovery was almost complete at roughly one year after diagnosis and beginning of treatment. The structural healing of the macular hole was concomitant with a strong improvement of visual acuity and the disappearance of metamorphopsia. The present findings are discussed in the light of a synergism shown at neuronal level between light and sound in the presence of phytochemicals to stimulate autophagy and promote proliferation and neuronal differentiation of retinal stem cells.

Key words

Light therapy • sound-sensitive retina • retinal stem cells • autophagy • macular hole • retinal degeneration • traumatic retinal injury • neurorepair.

Corresponding author: Professor Francesco Fornai, Human Anatomy, Department of Translational Research and NewTechnologies in Medicine and Surgery, University of Pisa, Via Roma 55 – 56126 Pisa (Italy), Tel: +39 050 2218667, and IRCCS Neuromed - Email: francesco.fornai@unipi.it; francesco.fornai@neuromed.it

Introduction

The neurobiology of the retina is key to understand which mechanisms may trigger and sustain degeneration and neuronal recovery. In this way, the retina is an ideal target for quickly probing translation of non-invasive therapeutic strategies to prompt future therapeutic planning. Among innumerous biochemical cascades, which regulate neuronal integrity in the retina, the autophagy machinery owns a seminal role to maintain cell survival and sustain retinal anatomy including plasticity. This is in line with the involvement of an impaired autophagy in producing a variety of retinal disorders (Pinelli et al., 2020b; 2020c; Intartaglia et al., 2021). In its essential definition, autophagy is involved in the clearance of misfolded proteins and damaged mitochondria. These in turn accumulate within retinal cells, in the course of a number of retinal degenerative disorders (Blasiak et al., 2014; Kaarniranta et al., 2020; Nita et al., 2020; Bilbao-Malavé et al., 2021; Yako et al., 2021). The involvement of autophagy is recently postulated to cover the mechanisms of regeneration which occurs as maturation phenomena following chronic degenerative conditions and sudden injuries as well. In fact, in CNS disorders an altered autophagy is seminal to promote chronic degenerative conditions (Ravikumar and Rubinsztein, 2004; Rubinsztein et al., 2005; Fornai et al., 2008a; 2008b; Castino et al., 2008; Ferrucci et al., 2008; Isidoro et al., 2009; Madeo et al., 2009), while sustaining maturation phenomena following acute brain injury as it happens during brain ischemia and epilepsy-induced brain damage (Wang et al., 2022a; 2022b; Zhang et al., 2021; Biagioni et al., 2021; Xiao et al., 2021). This is also the case of those area, which surround a trauma within the CNS, which integrity is recovered also depending on the autophagy status (Aruri et al., 2022; Chen et al., 2022). In fact, the nervous tissue, which surrounds an acute brain injury represents a sort of *penumbra*, where cells are borderline to recover or degenerate. In the process of sustaining neuronal survival, autophagy is key in removing cell components, which accumulate due to a defective metabolism. Nonetheless, recent evidence indicates that autophagy stimulation may also sustain a recovery process, which depends on the stimulation of stem cells. In fact, stem cell in the CNS are strongly stimulated to proliferate and differentiate towards a neuronal phenotype following autophagy activation (Chang et al., 2020). In recent reports we provided evidence showing that autophagy activation may sort protective effects and promote anatomical and functional recovery in the retina affected by chronic degeneration (Pinelli et al., 2020b, 2020c; 2020d; 2021a; 2021b). In fact, the autophagy status of a number of retinal cells is key in promoting the integrity of the retina. This the case of the retinal pigment epithelium (RPE), where autophagy acts at the RPE cell domain which touches the choroid Bruch's membrane (Blasiak et al., 2014; Kaarniranta et al., 2020; Sethna et al., 2021). Similarly, the RPE domain towards photoreceptors is affected as well by the autophagy status. In fact, abnormal deposits between the RPE and photoreceptors may be induced by altered autophagy (Pinelli et al., 2020c; Wu et al., 2021; Kim et al., 2021; Shijo et al., 2021). In this way, even focal alterations in the autophagy machinery affecting subcellular domain may play a role in conditioning retinal integrity (Pinelli et al., 2020c). Again, the ability of autophagy to alter the trafficking of exosomes across various retinal layers may explain the progression of autophagy-dependent beneficial or deleterious effects across the whole retina as inferred by pre-clinical studies (Pinelli et al., 2020c; 2021a; 2021b). Various autophagy-mediated pathogenic mechanisms for retinal degeneration are implicated. As previously hypothesized, Pinelli et al. (2020c), autophagy alterations may lead to several effects in the retina such as: (i) a deficiency of retinal protection against oxidative stress, free radicals and mitochondrial alterations; (ii) a loss of ability to counteract an overload of lipids, glycated products, and amino acids; (iii) a loss of stimulation of retinal regeneration through an impairment of specific niche of stem cells; (iv) an alteration of the blood–retinal barrier, which may no longer preserve retinal cells from circulating toxic species; (v) a loss of regulation of the retinal immune response, which may trigger an abnormal inflammation; (vi) a loss of polarity within the retina concerning metabolism and cell-to-cell communication; (vii) abnormal production of exosomes which may not protect the retinal tissue or may promote the occurrence of altered retinal plasticity (Pinelli et al., 2021a). Muller cells and specific retinal neurons undergo similar autophagy-dependent alterations. Thus, autophagy activation is recently emerging as a hot topic to study neuroprotective mechanisms in chronic retinal neurodegenerative disorders (Kaarniranta et al., 2022). Similarly, given the parallelism between traumatic and chronic degenerative conditions, the activation of autophagy in the course of traumatic CNS injury is thought to produce a strong activation to elicit functional and anatomical recovery (Movahedpour et al., 2022; Zhang et al., 2022; Filippone et al., 2022; Xu et al., 2022; Kanno et al., 2022). This is partly based on the induction of stem cells (Xu et al., 2021; Maiti et al., 2019; Ceccariglia et al., 2020; Hwang et al., 2021). The autophagy-dependent activation of stem cells may promote recovery in the retina as well (Usategui-Martín et al., 2022). Therefore, it is tempting to elicit autophagy via non-invasive stimuli in the course of mechanical/traumatic damage to the retina. Recently, a powerful effect of combined autophagy-inducing photomodulation by light therapy and its combination with autophagy inducers phytochemicals was described (Pinelli et al., 2021). Since light at specific wavelengths strongly activates autophagy (Suárez-Barrio et al., 2021), this is expected to synergize with establish autophagy activators such as specific phytochemicals (McCarty, 2022). The former represents the natural light, which depending on the specific wavelengths, apart from generating the process of vision, induces autophagy and it is key in removing mitochondria and altered proteins (Pevna et al., 2021; Stefenon et al., 2021; Yang et al., 2021). We recently probed a treatment based on translational evidence, which combines physical and chemical remedies, such as light exposure and nutraceuticals, according to what it was defined as "Lugano's protocol" (Pinelli et al., 2020c), which was hypothesized to converge in upregulating the autophagy status within the retina. This approach produced a remarkable regression of anatomical and visual impairment in a case of a degenerative retinal disorder (Pinelli et al., 2021c). In detail, in search for a potential synergism in inducing autophagy within the retina, a combined therapeutic approach was tempted in which nutraceuticals were administered, following pulses of different light owing different wavelengths. In recent studies the effects of light on promoting retinal integrity was shown to be enhanced by a combination of acoustic stimuli (Tonti et al., 2021). Therefore, in an effort to induce autophagy through the synergism of natural physical and chemical stimuli, the present study assessed the combined effects of light and sound to produce autophagy activation following such a photo-biomodulation, sound and light were combined with the same specific phytochemicals in a case of macular hole. Such a novel approach is defined here as "Advanced Lugano's Protocol" (ALP).

In detail, the background of the present study is based on the powerful effects of autophagy activation on the recovery from traumatic neuronal damage. Therefore, in an effort to synergize the effects of those mechanisms which elicit autophagy, in the present study we combined autophagy induction through light therapy with autophagy activating phytochemicals in a foveal damage produced by vitreal mechanical traction which generated a hole in the foveal region. The study was implemented by pulsatile sound stimulation based on the recent evidence that autophagy and retinal stem cells stimulation induced by light exposure is amplified by acoustic bio-feedback therapy. In fact pulses of sounds of various wavelengths stimulate tissue regeneration (Foglietta et al., 2015; Zhou et al., 2019; Xia et al., 2022), and such an effect is largely grounded on the stimulation of stem cell via an upregulation of autophagy (Wang et al., 2019). Altogether this evidence temped us to probe the efficacy of a non-invasive multimodal physico-chemical approach to promote substance recovery and anatomical re-organization in a case of idiopathic macular hole which was treated according to ALP.

Material and Methods

Patient presentation

A healthy 79 years old woman undergo an eye visit in January 2021, when she was diagnosed with an idiopathic foveal hole. The patient did not carry additional significant disorders and otherwise she was in good healthy conditions. Nonetheless, she reported allergic reactions to Plasil and Flour. At diagnosis she was tested for visual acuity by using the Snellen eye Chart with optotype. When she was visited she was consulted with other specialists who confirmed the diagnosis of Macular hole in OD. Following such a diagnosis she was proposed a surgical treatment of the macular hole by using vitrectomy and peeling. At the time of diagnosis her visual acuity in OD was very poor, being the natural visual acuity, without correction of 1/10 and following best correction visual acuity did not exceeded 4/10. At the time of diagnosis she suffered from a slight central metamorphopsia (Amsler grid). The diagnosis of idiopathic macular hole caused by vitreal degeneration with a traction on the foveal part of the retina was made.

Following a completed informed consent she was aware and willing of trying a pulsatile stimulation with sound and light (photo-biomodulation) with an oral intake of phytochemicals. In detail, the information provided to the patients specified the non-invasive nature of the procedure, the risks and the benefits, and potential alternative (mainly surgical) options for the treatment of idiopathic macular hole. During and after the treatment period, the patient was evaluated by using optical coherence tomography (OCT) to assess the time course of the volume and the integrity of surrounding or inner tissue replacement of the macular hole occurrence of drusen and the retinal topography was provided to calculate the thickness of the various layers of the retina and the empty area in the hole. OCT, which provides an anatomical measurement of the foveal tissue, was accompanied by subjective visual tests. These were carried out mostly with the aim to assess the visual functions of the fovea, which provides visual acuity. A total of four subjective tests were applied. The Jaeger Chart test and the Snellen Chart test to score the best corrected visual acuity for near and far (BCVA); the Amsler grid test was used to test the occurrence of visual distortion (metamorphopsia, wavy lines), while the Pelli-Robson Chart test was applied to measure contrast sensitivity. Each test was repeated 3 times in order to express the mean value.

Treatments

The patient was exposed to session of a non-invasive therapy of low sound, near-infrasound frequency acoustic stimulation consisting of sound waves in a range between 20 and 174 MHz, which were applied at progressive increasing frequency up to 174 MHz. At 174 MHz stimulation was continued and frequency was kept steady. The stimulation was carried out when the patient was sitting on a reclined chair with sound stimulation periods lasting 90 sec. These periodic stimulation was repeated within the same day according to a pattern of 7 periods of acoustic stimulation each one lasting 90 sec, which was delivered at an interval of 30 sec apart (interval of acoustic silence) from the following 90 sec period of acoustic stimulation, for a total duration of 15 min. The subjective intensity (decibel scale) of the acoustic stimulus, was dependent on the frequency and it was delivered in the range between 20 and 30 dB. The sound application was delivered through commercial Beats headphones (in plastic). At the end of acoustic stimulation, following a 2 months interval OCT was carried out along with examination of visual acuity. Since result obtained with sound stimulation were partial, based on recent literature (Pinelli et al., 2022), it was decided to combine sound with Photobiomodulation in the same single session and to add phytochemicals as natural nutraceutical waves.

Photobiostimulation (PBM)

Based on partial data obtained with acoustic stimulation and according to the proof of concept of combining light and sound therapy the treatment protocol was implemented by adding to sound a session of photobiomodulation as recently reported (Pinelli et al., 2021) under the name of Lugano's protocol. Briefly, PBS was carried out according to the "Lugano protocol". In detail, PBM was applied at 60 sec following sound stimulation. PBM consists of 9 sessions of light exposure. In fact the patient was applied a total of 9 sessions of light stimulation. Each session was composed on exposures to various wavelengths. Light exposure was carried out for one month approximately 3 sessions per week. Three different wavelengths were used in each session, specifically, 590 nm, 660 nm, and 850 nm, which correspond to yellowish amber red, red, and infra-red, respectively. Light was produced by specific diodes (LEDs, Valeda Light Delivery System). The light produced by the led passed through a beam owing a diameter of 30 nm (nominal) with a direction which was parallel at treatment plane. In fact, beam orientation was strictly parallel to the treatment plane and horizontal axis. Each session consisted of 250 seconds light exposure. When the 590 nm light was administered, the power per unit area was set at 65mW/cm2; for 660 nm light the power per unit area was 8mW/cm2, while the light beam of 850 nm was applied with a power per unit area of 8 mW/cm2.

Various light wavelengths and light power were combined in each session different according to the following scheme, SESSION 1 35 seconds of pulsatile yellow/amber light (590 nm wavelength) and infra-red light (850 nm wavelength) patient's eyes wide open. SESSION 2 of continuous 90 seconds red light (660 nm wavelength), patient's eyes wide shut. SESSION 3 pulsatile 35 seconds of yellow/amber light (590 nm wavelength) and near infrared light (850 nm wavelength), with patient's eyes wide shut. SESSION 4: continuous red light (660 nm wavelength) for 90 seconds patient's eyes wide shut. These sessions were repeated 3 week per 3 weeks during 1 month total time of treatment.

Nutraceuticals

After combining sound+PBM patient started nutraceuticals at home. Nutraceuticals were supplemented to the patient's diet which was not modified otherwise. Each compound was supplemented in the form of a powder in equal amounts of the extract from Tagetes erecta, lutein; an extract of Polygonum cuspidatum, resveratrol; Vaccinium myrtillus extracts also known as bilberry. The dose of these compound consisted of 6 g, daily 20 days a month for a total of six months.

Evaluation of anatomical integrity of the fovea and visual acuity

This was carried out by combining instrumental (anatomy, OCT) and subjective (visus) clinical test. The latter were applied at diagnosis (pre-treatment), after acoustic stimulation started (3 months) and following adding on the combined treatment of soung+light PBM+nutraceuticals at 6 and 10 and 15 months after diagnosis.

Optical coherence tomography (OCT)

Optical coherence tomography is the gold standard exam used to measure the anatomy of the retina and it works just fine for the evolution of the macular hole. In fact, this exam provides a direct visualization and measurement of the foveal/macular hole and the amount of tissue which surrounds it or covers it or fills it. As well as derangements or recovery of specific retinal layers. OCT consists of a non-invasive imaging procedure, which is based on visible light waves, being reflected from different layers of the retina and external choroid structures. This method also allows to measure the amount of altered flatness produced by a macular hole and the potential inflammatory exudate which may cover it.

Retinal topography

It specifically measures the thickness of the retinal layer including the macular region and it allows to measure the hole which covers such a retinal field. It is useful to detect the specific site where an alteration of the planar arrangement is produced under the mechanical pressure of underlying structures (the macular hole in this case). It is obtained by combining the OCT technique along different axis.

Visual tests

Since as recently published, the occurrence of a macular hole imped visual acuity, the careful detection of visus is fundamental in establishing the subjective clinical course of the disease. Therefore, clinical subjective routine test to assess visual ability are mandatory to validate the disease course and potential disease modifying treatments. In line with this, the patient had progressive subjective tests to measured visual function. The tests were repeated 3 times in order to express the mean value. The tests here used include the following: the Jaeger Chart test, the Snellen Chart test, the Amsler grid test and the Pelli-Robson Chart test (Contrast sensitivity test).

Jaeger Chart test

The Jaeger Chart test was used to determine the near best corrected visual acuity (BCVA). The apparatus consists of a chart reporting short written text blocks of different sizes, Snellen eye chat with optoptype. The chart is held at a specified reading distance (35 cm) and the patient is asked to read the smallest block of lines she can focus from the biggest block (J10 or 1/10) to the smallest block (J1 or 10/10). If the patient read a specific block of letters without squeezing, that block is considered to be visualized correctly. Different blocks were shown during each detection in order to avoid learning words by heart. The score J10 to J1 may also be expressed in percentage (10% to 100%) where 100% is the maximum visual acuity for near (J1).

Snellen Chart test

The Snellen eye Chart test was used to determine the far best corrected visual acuity (BCVA). A retro-illuminated wall-mounted Snellen chart is used with the patient standing at 6 m from the chart (Johnson et al., 1998; Chen et al., 2014). The chart includes red and green color bars for an easy and helpful place to start administering the test. There are 10 rows of decreasing size at a pre-determined distance. The patient is asked to read 5 letters per row (from row 1/10 to row 10/10). If the patient read at least 3/5 of letters in a specific row and 1 or 2 letters of the subsequent row, the previous row is considered to be visualized correctly. Different letters are shown during each detection in order to avoid learning letters by heart. Even in this case for measuring far visual acuity, the score 1/10 to 10/10 is converted in percentage (10% to 100%) where 100% is the maximum far visual acuity (10/10).

Amsler Grid test

It is a grid of horizontal and vertical lines used to monitor a person's central visual field. It is a diagnostic tool that, allows detecting visual disturbances caused by changes in the retina, particularly the macula, as well as the optic nerve and the visual pathway to the brain. Amsler Grid test usually helps detecting defects in central 20 degrees of the visual field. The apparatus consists of a white square-shaped grid divided by horizontal and vertical black lines in approximately 20 small squares in each side of the grid. A central black dot is present for orienting the sight. The illumination of the chart is kept steady and optimal to allow the best resolution. The grid is kept at least 33 cm far from the eye. The patient is asked to close one eye and each eye is tested separately. In patients with altered vision the lines of the square appear distorted, otherwise they look parallel (Su et al., 2016). Patients with macular disease typically observe line distortion appearing as wavy, interrupted or disturbed lines with some lines may be missing in the subjective report. In this study, the score was altered only before treatment was started, scoring is given as follows: 10 (0 wavy lines); 9 (1 wavy, interrupted, disturbed horizontal or vertical line); 8 (2 wavy, interrupted, disturbed horizontal or vertical line); 7 (3 wavy, interrupted, disturbed horizontal or vertical line); 6 (4 wavy, interrupted, disturbed horizontal or vertical line). 5 (wavy, interrupted, disturbed horizontal or vertical line); 4 (6 wavy, interrupted, disturbed horizontal and vertical line); 3 (7 wavy, interrupted, disturbed horizontal or vertical line); 2 (8 wavy, interrupted, disturbed horizontal or vertical line); 1 (9 wavy, interrupted, disturbed horizontal or vertical line).

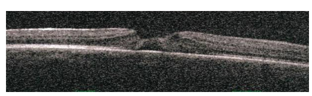
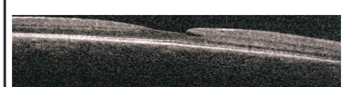
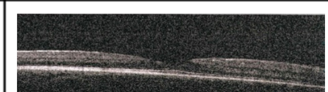
Pelli-Robson Chart test

Pelli-Robson Chart test measures the contrast sensitivity defined as the ability to perceive slight change in luminance between regions, which are not separated by clear-cut defined borders. The chart is composed of letters (6 in each horizontal line) arranged in groups, whose contrast varies from high to low. The patient read the letters, starting from the highest contrast, until she is unable to read two or three letters in a single group. Each group has three letters owing the same contrast level, so there are three trials per each contrast level. The score is based on the contrast of the last group in which two or three letters are correctly red. A Pelli-Robson score of 2.0 indicates normal contrast sensitivity, a score of less than 1.5 is consistent with visual impairment and a score of less than 1.0 represents visual disability.

Results

A 79 years-old patient, which was suffering from vitreal degeneration developed idiopathic macular hole with marked substance loss and atrophy which was complicated with a retinal damage which was due to a mechanical traction on the foveal region.

At the time of diagnosis, the vision was markedly affected. In fact the Amsler test measured the occurrence of wavy lines, central, close to the central black point in horizontal vision which became impaired for the presence of central slight metamorphopsia (Figure 1) and a dramatic loss of far visual acuity which was evident in a lens-corrected BCVA of 20/160 and a best visual acuity for near of J2 following correction as shown in Table 1. The anatomical structure of the macula was seriously altered at OCT (Figure 2) approaching a stage 3/4 macular hole. Contrast sensitivity at the time of diagnosis was 1.10 At this stage, the volume of the macular hole detected at retinal topography was measured as 333 mm^3.

	Parameters before treatment	Parameters at 6 months	Parameters at 10 months
BCVA For near	20/160 with lenses J2 with lenses	20/63 with lenses J2 with lenses	20/25 with lenses J1 with lenses
Contrast sensitivity	1.10	1.8	2.0
Amsler Test	Wavy lines, central, near the central black point in horizontal	Negative	Negative
Volume of Foveal hole	333 μm^3	298 μm^3	227 μm^3
Macular Topography			

Tab. 1 – The table shows the visual acuity, contrast sensitivity, visual distortion and provide a rough anatomical correlate at diagnosis and at selected time intervals at 6 and 10 months. Data indicate a reversal of the anatomical and visual alterations produced by the macular hole following a combined therapy of sound+light+nutraceuticals.

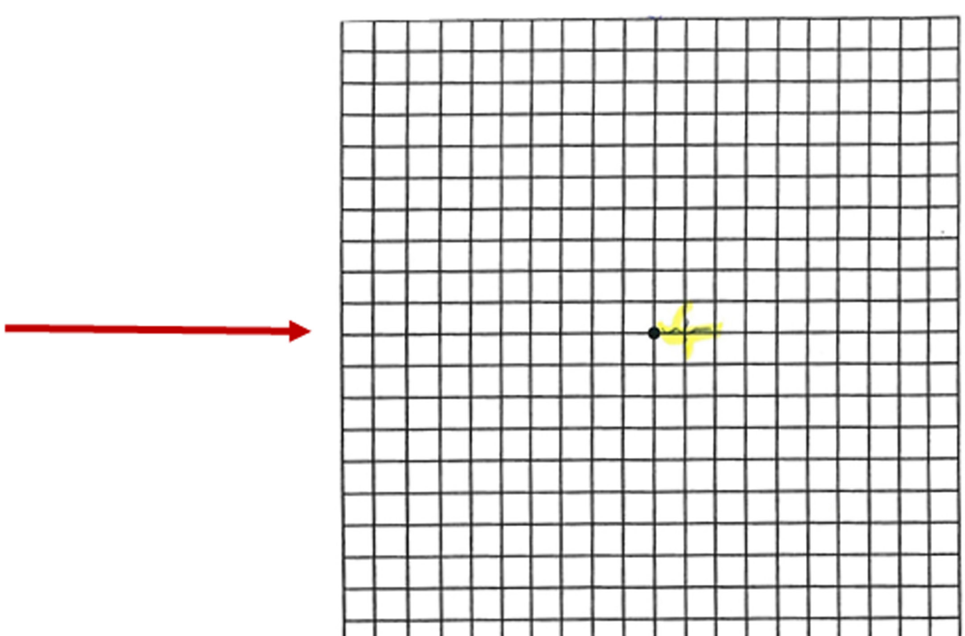
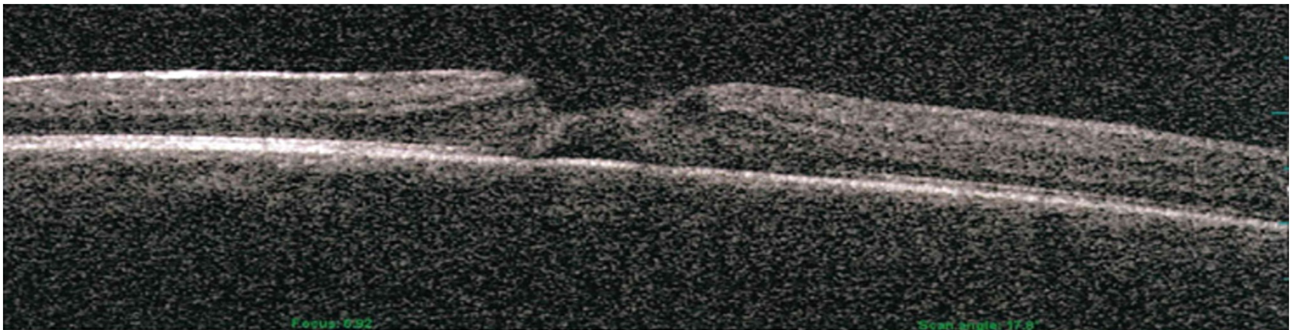


Fig. 1 – Amsler grid at diagnosis. The figure shows a slight central metamorphopsia.



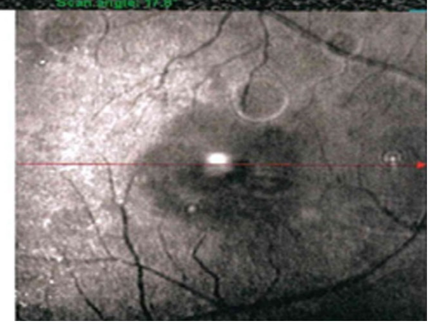
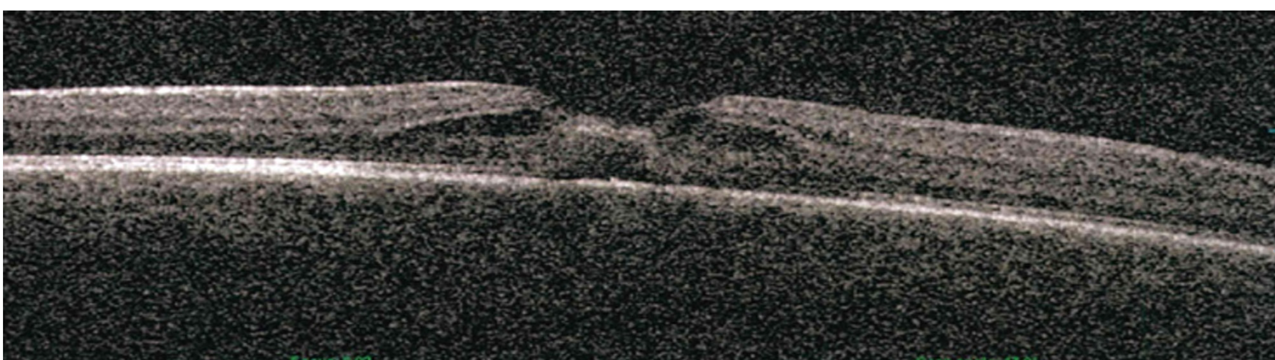


Fig. 2 – Optical coherence tomography (OCT) at diagnosis. The OCT shows a severe macular hole which is present at diagnosis.



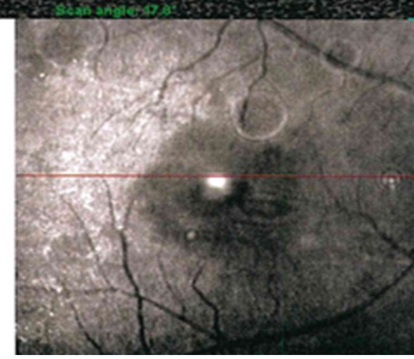


Fig. 3 – Optical coherence tomography (OCT) at 3 months following sound stimulation. The OCT shows a slight recovery following sound stimulation.

In this condition the patient started a simple sound therapy which was providing a slight recovery of the retinal anatomy at OCT (Figure 3). At this stage the patient did not experience any significant subjective visual improvement and metamorphopsia although mild was still present. At this stage the retinal anatomy did not improve very much although the external retina (Figure 3) was thicker and the hole in the inner retina was less deep. At this time patient started combined acoustic and light modulation (PBM) for 1 month which was followed by nutraceuticals. At 6 months following diagnosis there was a significant improvement of retinal anatomy (Figure 4) visualized at OCT with a loss of metamorphopsia reported by the patient as the absence of central distortion. There was a

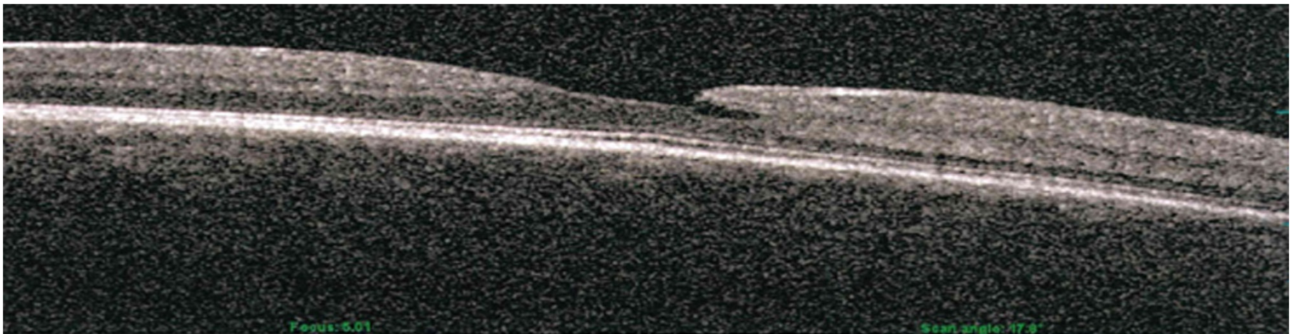
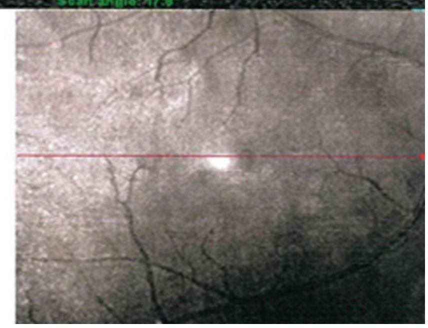



Fig. 4 – Optical coherence tomography (OCT) at 6 months following treatment. The OCT shows a marked improvement with the healing of the macular hole following added therapy with photobiostimulation (PBM, light and sound) and nutraceuticals.

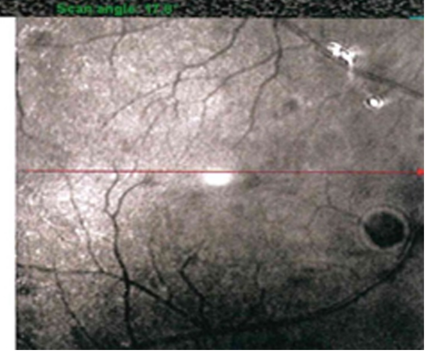
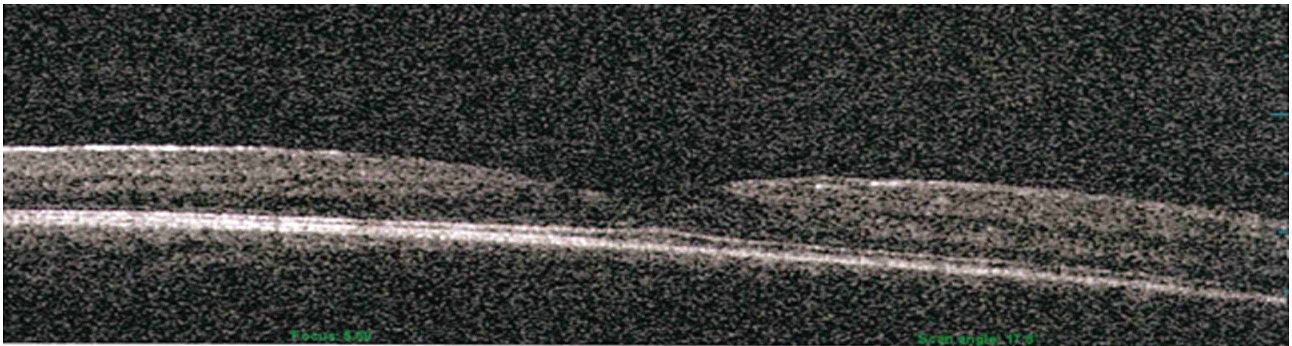



Fig. 5 – Optical coherence tomography (OCT) at 10 months following treatment. The OCT shows that the healing is almost complete concerning the macular hole.

remarkable increase in contrast sensitivity, which rose up to 1.8, while the improvement for far visual acuity was improved up to 20/63 with lenses and the best visual acuity for near rose up to J2 with correction. The patient perceived a slight improvement of visual acuity. At this time period a further decrease in the macular hole was measured as reduced volume at retinal topography (298 mm^3, Table 1). At the following follow up, at 10 months following diagnosis the retina anatomy was remarkably improved with a healing of the macular hole which was almost complete (Figure 5) and the patient referred a clear improvement of vision which was scored as 20/25 at BCVA after lens correction. The visual acuity for near was finally J1. At 15 months from diagnosis the recovery of the macular



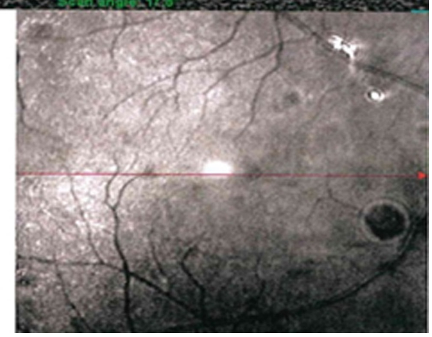


Fig. 6 – Optical coherence tomography (OCT) at 15 months following treatment. The OCT shows a steady recovery without any relapse from the macular hole. The combined treatment of sound, PBM and nutraceuticals, produced a full and steady recovery at the macular hole. Such an anatomical recovery was accompanied by a remarkable recovery in visual acuity.

structure was complete and steady with a slight rarefaction within the outer nuclear level (Figure 6). The visual acuity was steadily recovered with an absence of linear distortion.

Discussion

In the present study, a patient suffering from a foveal hole produced by a mechanical traction of the vitreal body in the foveal region of the retina was administered a combined therapy based on phytochemicals and light stimulation which was proven already to be beneficial in a case of retinal degeneration (Pinelli et al., 2021). This treatment was implemented by acoustic intermittent stimulation based on the evidence that pulses of sounds synergize with light in stimulating stem cells of the retina (Tonti et al., 2021). The combined administration of phytochemical and light/sound stimulation, is defined here as Advanced Lugano Protocol (ALP), produces a remarkable visual improvement, which is associated to a near complete anatomical recovery in the macular region.

Such a remarkable effect is unlikely to rely on spontaneous recovery of macular tissue and may depend on the combined physical and chemical stimulation provided by the ALP therapy. The site of action of ALP may extend to the region of corpus vitreous. In fact, as shown by Vogt et al. (2020), in the presence of full thickness macular holes a strong synthesis of autophagy dependent neurotrophic factor (such as ciliary neurotrophic factor and GDNF) takes place much more on the vitreal side than the inner retina, which surrounds a macular hole. This explanation would provide also the basis of a lack of relapse once the therapy was completed. In fact, it is likely that tissue regeneration may extend to cells of the vitreous beyond the border of the inner limiting membrane (Vogt et al., 2020). These cells would represent a sort of pre-macular cells present on the vitreous side of the hole.

Light is essential in providing the natural stimulus to generate the process of vision, while it is able to induce autophagy and neurotrophic effects (Pevna et al., 2021; Stefenon et al., 2021; Yang et al., 2021). The effects of light are strengthened by combined exposure to acoustic stimulation. In fact, a synergism may derive from a synesthesia, which may act just on retinal stem cells, when pulses of electromagnetic fields are coupled with pulses of acoustic energy. This was recently reported during training of patients suffering from central scotoma with acoustic bio-feedback. These patients experienced a progressive improvement of visual acuity, by moving fixation to eccentrically placed healthy area of the retina, which was primed to act as a "pseudo-fovea" (Tonti et al., 2021).

Thus, we probed the effects of a treatment based on translational evidence, which combines physical and chemical remedies, such as light/sound exposure and nutraceuticals, according to an amount and time of exposure which is defined here as ALP, which is now hypothesized to converge in upregulating the autophagy status within retinal cells and promote stem cells stimulation.

Increasing evidence shows that autophagy regulates the process of retinal repair and it is able to affect retinal stem cells. This recent literature is backed by solid data showing a powerful, autophagy-dependent, stimulation of retinal stem cells induced by different wave-lengths of natural light for administered at specific time intervals. Again, sound energy is strongly implicated as an autophagy activator as recently shown by a number of studies where mitochondrial (mitophagy) and protein clearance which represent the essential activity of autophagy are stimulated by short pulses of sound (Guo et al., 2019).

The effects of sound on autophagy in the retina and acoustic epithelium was a pioneer observation by Ganesan et al. (2008). In fact, these authors emphasize the properties of melanin, which is essential in the transduction and perception of sound in the inner ear, to be sensitive to sound stimulation. It is remarkable how melanin is essential in visual and photic activities of the retina as well as in the stimulation of retinal stem cells. Thus, it is not surprising that, when a photo-pigment is also a sound-sensitive pigment, its placement in the photic and acoustic epithelium sort dual effects on different sensory organs. In this way, recent evidence accumulates showing that sound stimulates stem cells (Wang et al., 2019) just like we reported for light. Again, as reported by Zeng (2019), such an effect is bound to autophagy activation and modulation of exosome trafficking, which is key in retinal plasticity (Pinelli et al., 2021b). The role of sound in inducing stem cells is wide and occurs in various tissues (Chiang et al., 2019; Zhou et al., 2019). In all cases, such an effect is definitely dependent on sound-induced autophagy activation (Li et al., 2020). This latter effect is so powerful that even sound induced trans-cranial transmission promotes the activation of autophagy within specific classes of neurons (Huang et al., 2021). As in the case of melanin, sound and light possess some intrinsic property in their form of energy, which is able to activate specific photo- and sound- sensitive protein, which converge in autophagy activation. In turn, this is key in promoting tissue repair (Xia et al., 2021) and fostering exosome-mediated cell-to-cell communication (Xia et al., 2022). Such a widespread influence of sound and light, and their synergism in activating the autophagy machinery to counteract retinal damage was exploited in a recent manuscript by Liu et al. (2022). In this study, Authors demonstrated how intravitreal gene therapy aimed at up-regulating autophagy activity was able to mitigate retinal degeneration. This recalls how the integration of acoustic and visual stimuli, apart from being essential in supra-modal cortical areas (Recanzone, 2009; Ricciardi et al., 2009; Zilber et al., 2014;) may occur already within the retina to produce a synergism, which impinge on retinal stem cells to produce the effects reported by Tonti et al. (2021). The present data may be interpreted according to various concomitant effects; in any case, the synergism at the autophagy machinery and stem cells stimulation produced by light and sound and phytochemicals should be seriously taken into account.

In fact, in the course of the present study, we do not have any direct evidence about which molecular mechanism underlies such a synergistic effect; nonetheless, translational studies and clinical report lead to hypothesize a powerful modulation of retinal structural recovery, which is based on trophic effects produced by phytochemicals and light exposure when combined with pulsed of acoustic stimuli. In fact, specific wavelengths increase proliferation rate of retinal stem cells over four-fold compared with that measured in baseline conditions (Wang et al., 2019). This phenomenon occurs within retinal stem cells, which do receive either blue or red stimulation. This phenomenon purely depends on the impact of light and it is highly dependent on the wavelength, which stimulates the retinal stem cells. In fact, in the anterior part of the retina a rich stem cell niche occurs, which promotes a wavelength-dependent cell proliferation and cell differentiation in the retinal neurons and glia. In detail, blue wavelengths induce a preferential transformation of stem cells in glia; in contrast, infrared light stimulation produces the neuronal differentiation of these stem cells (Wang et al., 2019). These effects are induced by pure light, which acts as an archaic vision-independent, photic plasticity. This requires pure physical energy in the aspect of oscillatory phenomena of electromagnetic fields as the appropriate stimulus. This brings intriguing questions on the potential synergy in the form of synesthesia, which may act on retinal stem cells when pulses of electromagnetic fields are coupled with pulses of vibrational acoustic energy. In fact, as recently reported, training of patients suffering from central scotoma with acoustic biofeedback improves visual acuity. In these conditions the retinal fixation is moved from the fovea to eccentrically placed healthy area of the retina, which are primed to act as a "pseudo-fovea" (Tonti et al., 2021). In keeping with plasticity induced by pure light, which occurs depending on pulses of electromagnetic wavelengths, it is remarkable that both blue and red light need to be applied according to intermittent (vibrational) patterns. In fact, as originally demonstrated by Wang et al. (2019), when photic stimulation is applied according to a specific timing (45 min for 5 consecutive days) both glia and neurons phenotypes may be induced based on the specific wave-length. Such a powerful effect of light on retinal regeneration shed a practical perspective in patients, who improved following photo-stimulation as reported in a recent manuscript (Pinelli et al., 2021c). The remarkable evolution of the visual processing represents the ultimate step, which is triggered by light and photoreception. When studying archaic nervous systems the impact of light is rather represented by mere photic stimulation, which lacks the ability to provide information concerning the visual scenario, which is commonly defined as visual field. This implies "neither shapes nor color detection, neither object nor movement perception but mere light, which variably contrasts and brakes in the shadow" (Pinelli et al., in press, 2022).

The natural progression of macular holes is reported extensively by Chew et al. (1999). In this study, where follow up was carried out in 122 patients, authors report a negative progression of macular holes. In these patients the common course of the macular hole once induced is stable (40.9% of the patients) or it worsens in 34% of the patients. A spontaneous regression was observed only in 3/122 patients and it was never present before 6 years follow up. Thus, a complete anatomical and visual regression was never reported at 1 year following non-surgical, non-invasive treatment.

Stino et al. (2022) recently described the time-course following partial thickness macular holes. In this study epi-retinal proliferation was associated with worsening of visual acuity, which was accompanied by neuro-retinal tissue loss with wider foveal cavities and thinner foveal floors. During the follow-up, visual acuity tends to be stable, independently by the severity of macular holes.

The surgical approach to macular holes shows a high frequency in the closure, which occurs following surgery. However such an outcome strongly depends on which kind of macular hole (staged based on the size) is approached by surgical vitrectomy (Kim et al., 2022). The non-surgical improvement of macular holes was very recently described by Mansour et al. (2022). These authors described the recovery time following full-thickness macular hole produced by a trauma due to vitreous-macular traction. When considering the recovery of a continuous outer nuclear level the mean time exceeds 6 months, and 9 months are needed to rebuild the external retinal outer membrane. The mean recovery when considering the regeneration of the inner or outer retinal segments occurred at roughly 13 months following the hole onset. Although most report indicate the lack of progression in the loss of visual acuity following idiopathic macular holes, the recovery of the anatomical structure does not occur concerning inner retinal defects. Thus, macular hole are reported to be stable or undergo a deleterious process which leads to a further loss of integrity during a 12 months follow up (Goel and Skula, 2021). Thus, the recovery of retinal tissue observed and measured in the present study, which took place in such a short interval, just exceeding one year, it is remarkable and calls for some pro-regenerative effects.

Light and sound exposure produces activation of archaic brain circuitries, which are the final common pathways for different kind of stimuli. Within such a rudimental anatomical recipient, light merges with sounds, and pain and multiple sensory systems to adjust physiology and behavior of the human body. Within these circuitries both stimuli stimulate autophagy just like occurring in the specific sensory apparatus.

Within the retina, although some studies reported that autophagy mostly acts between the retinal pigment epithelium (RPE) and the choroid Bruch's membrane (Blasiak et al., 2014; Kaarniranta et al., 2020; Sethna et al., 2021), other studies indicate the relevance of autophagy between the RPE and photoreceptors (Pinelli et al., 2020c; Kim et al., 2021; Shijo et al., 2021; Wu et al., 2021). The occurrence of retinal degeneration is postulated to derive from focal subcellular autophagy impairment at various retinal levels (Pinelli et al., 2020c), and it may serves to distinguish between different phenotypes of retinal degeneration (Pinelli et al., 2020c; Kim et al., 2021; Shijo et al., 2021; Wu et al., 2021). In fact, according to the hypothesis of an exosome–dependent trafficking of misfolded proteins towards different poles of the retina, as inferred by pre-clinical studies (Pinelli et al., 2020c; 2021a; 2021b), various autophagy-mediated pathogenic mechanisms within the retina may be implicated.

In detail, the updated retinal microanatomy indicates that RPE as well as Muller cells act as a pivot to grant outer and inner retinal metabolism (Gass, 1972; Ambati and Fowler, 2012; Boulton and Dayhaw-Barker, 2001; Bonilha, 2008; Kozlowski, 2012). In this way, the sub-cellular organelles and biochemical responses to varying insults depend on these cells to produce the onset and progression of retinal damage. As summarized in Pinelli et al. (2020c) these include (i) a failure in retinal protection from oxidative and mitochondrial stress; (ii) a loss of ability to cope with lipid, glycogen, and protein overload; (iii) impaired renewal; (iv) a loss of the outer blood–retinal barrier; (v) the occurrence of abnormal inflammatory/immune response; (vi) a loss of retinal polarity, thus reverting the metabolic flow towards photoreceptor and from the choroid; (vii) accumulation via an exosome dependent process of extracellular waste material including proteins advanced glycation end products (AGEs) and lipids (Pinelli et al., 2021a). All these effects appear to be under the influence of the autophagy status within the retina.

Therefore, in the present study, we wish stimulate retinal autophagy by combining various approaches to progress the outcome of a structural loss of retinal integrity up to a total reversal of anatomical derangement in the macula as detectable at OCT. Thus, in search for a potential strengthening of an autophagy-based disease modifying mechanism, a combined therapeutic approach named ALP, is carried out here, where the very same nutraceuticals were administered in combination, following two natural physical ongoing stimulation of the retina. The latter represents the natural light and sound, which depending on the specific wavelengths, apart from providing the natural stimulus to generate the process of vision, is able to induce autophagy and it is key in removing mitochondria and altered proteins (Pevna et al., 2021; Stefenon et al., 2021; Yang et al., 2021). This approach is carried in a patient carrying a macular hole to evaluate to which extent the anatomical and clinical alterations were modified (either worsened or halted, or even reverted) during and after a 15 months treatment. The molecular mechanisms operating during such a phenomenon are extensively discussed in the light of the interactions at biological level between exposure to long wavelengths and the effects of nutraceuticals to synergize on the autophagy status to counteract the mechanisms generating the neurobiology of retinal integrity and anatomical architecture.

When studying archaic nervous systems, the impact of light is rather represented by mere photic stimulation, which lacks the ability to provide information concerning the scenario, which is commonly defined as peripheral visual field. This implies neither shapes nor color detection, neither object nor movement perception but mere light, which variably contrasts and brakes in the shadow. In this way, light exposure produces activation of archaic brain circuitries, which are the final common pathways for different kind of stimuli. Within such a rudimental anatomical recipient, light merges with sounds, and pain and multiple sensory systems to adjust physiology and behavior in the human body.

The powerful plastic effects of light are evident already in the retina itself since pioneer papers during the mid'70s up to recent reports. This concept, is well established concerning intrinsic retinal circuitries connected with vision (Berry, 1976; Rose, 1977), and during retinal degeneration (Pinelli et al., 2020a; Pinelli et al., 2021a; 2021b; 2021c; Strettoi et al., 2022) including plastic changes in the retinal pigment epithelium (Pinelli et al., 2020b). This is now strengthened by evidence showing that specific wavelengths increase proliferation rate of retinal stem cells over four-fold compared with that measured in baseline conditions (Wang et al., 2019). This phenomenon occurs within retinal stem cells, which do receive either blue or red stimulation. This phenomenon purely depends on the impact of light and it is highly dependent on the wavelength, which stimulates the retinal stem cells. In fact, in the anterior part of the retina a rich stem cell niche occurs, which promotes a wavelength-dependent cell proliferation and cell differentiation in the retina itself. These cells are shifted towards glia by blue wavelengths, while red light promotes a neuron phenotype (Wang et al., 2019). Pure light in the form of pure oscillatory physical energy of electromagnetic fields is the appropriate stimulus. This brings intriguing questions on the potential synergy in the form of synesthesia, which may act on retinal stem cells when pulses of electromagnetic fields are coupled with pulses of acoustic energy. In fact, as recently reported training of patients suffering from central scotoma with acoustic biofeedback can progressively improve visual acuity, by moving fixation to eccentrically placed healthy area of the retina, which is primed to act as a "pseudo-fovea" (Tonti et al., 2021). This is in line with the concept investigated by Ricciardi et al. (2009) based on investigation of cortical brain network sub-serving perception in blind people: how much do we need light and vision? In keeping with plasticity induced by pure light, which occurs depending on pulses of electromagnetic wavelengths, it is remarkable that light, which stimulates the stem cells to form neurons need to be applied according to intermittent patterns. In fact, as originally demonstrated by Wang et al. (2019), when the photic stimulation is applied according to a specific timing (45 min for 5 consecutive days) both glial and neuronal phenotypes are induced just depending on the specific wave-length. Such a powerful effect of light on retinal regeneration shed a practical perspective in patients who improved following photo-stimulation as reported in a recent manuscript (Pinelli et al., 2021c). The remarkable evolution of the visual processing represents the ultimate step, which is triggered by light and photoreception. When studying archaic nervous systems the impact of light is rather represented by mere photic stimulation. In this scenario, the movement perception is likely to belong to sound energy rather than visual processing as it occurs in blind people (Ricciardi et al., 2009). Thus, we may speculate that sound comes first and organizes the CNS accordingly. In this way, light exposure just impinges in the activation of archaic brain circuitries, which are the final common pathways for different kind of stimuli. Within such a rudimental anatomical recipient, light merges with sounds, and pain and multiple sensory systems to adjust physiology and behavior in the human body. In line with this, the effects of light on promoting retinal integrity is magnified by a combination of acoustic stimuli (Tonti et al., 2021). The pulsatile sound stimulation promotes autophagy and retinal stem cells stimulation induced by light exposure is amplified by acoustic bio-feedback therapy. In fact pulses of sounds at various wavelengths stimulate tissue regeneration (Foglietta et al., 2015; Zhou et al., 2019; Xia et al., 2022), and such an effect is largely grounded on the stimulation of stem cell via an upregulation of autophagy (Wang et al., 2019). This is why in the present study it was probed the efficacy of a non-invasive multimodal physico-chemical approach to promote substance recovery and anatomical re-organization in a case of idiopathic macular hole which was treated according to ALP. Combining different stimuli sort a powerful effect on autophagy activation and it is likely to promote significantly the recovery after a traumatic neuronal damage. Therefore, in the present study sound was combined with light to sort a strong autophagy induction with light therapy and with autophagy activating phytochemicals following a retinal mechanical damage induced in the macular region by vitreous mechanical traction, which generated a hole in the foveal region.

In fact, the etymology of photoreceptor it is not necessarily related to visual perception but the elementary detection of electromagnetic fields without any visual implication. In this context, even archaic systems such as plants possess photoreceptors, and they are strongly modulated by light exposure. This is possible due to specific surface cells, which receive light and specific biochemical species, light sensitive molecules, which structure and activity is modified by specific wave-lengths and light intensities. The photo-pigments which respond to light are often responsive to oscillatory acoustic energy, as in the case of melanin.

When conceived within the frame of comparative biology the natural questions which rises up is the following: is such an ancestral role of mixed sound and light progressively lost in the evolution to be replaced by the visual and auditory systems? Or, this is rather preserved in the human retina? In line with this, is the presence of photosensitive molecules still relevant, aside from visual processing, in the photic effects produced by light exposure in the human CNS?

The maintenance of sound- and light-dependent responses in the retina, which occurs even in the absence of the CNS is a classic example of how conserved is the archaic organization of such a light sensitive epithelium. In fact, evidence indicates how within the retina a sub-class of neurons are able to sense light independently from image forming activities. This cell population corresponds to intrinsically photosensitive retinal ganglion cells (iPRGCs). These cells, which are identified for three decades (Freedman et al., 1999; Lucas et al., 2001).

Conclusions

The present manuscript reports a case of retinal hole, which greatly benefits from a combined exposure to broad visible wavelengths ranging between amber and near infra-red supplemented by pulses of acoustic stimulation and three nutraceuticals, lutein, resveratrol, and blueberry extracts (ALP). The benefit was evident in all altered visual symptoms and it was measured by an improvement of deranged retinal anatomy. In particular, it needs to be emphasized how the beneficial effects induced by PBM were further improved by adding a treatment with nutraceuticals. Such a near total recovery should be considered also in the light of disease severity. It is reasonable that the potential plasticity of the retina towards a recovery is likely to be higher when the disease is treated at early stages. On the other hand, the concept of plasticity being much more active in young compared with old people should encourage optimism in considering the advanced age of the patient. We might consider that, despite being 79 (and now 80) years old, the patient suffered from a form of macular hole, which possesses an intrinsic recovery. Nonetheless the remarkable effects observed here are quite unique and call for a substrate, which is mostly responsive to these potential disease-modifying treatments. The recovery of integrity is likely to ground on a wider range of functional and anatomical effects, which naturally occur in the retina.

In fact, the outcome of the present treatment, which is known as ALP, is more evident at morphological than clinical level. This is likely to rely on the lower threshold to detect retinal alterations by dedicated retinal morphology. Nonetheless, the improvement in vision was also remarkable. In fact, it is not surprising that biological substrates are sensitive to both chemical and physical stimuli mostly when considering the natural exposure of the retina as a gateway of the nervous system with the external environment. The physical energy in the facet of electromagnetic and sound waves can be both considered as natural physical stimuli, considering the sound-sensitive properties of retinal photo-pigments. Such a phenomenon does occur based on the ability of light and sound to synergize in triggering specific biochemical cascades within the cells of the retina. To make it simpler, our eyes provide vision since a physical energy in the form of specific electromagnetic wavelengths or quanta triggers specific molecules which also occur in the auditory system. It is not surprising that these stimuli enhance the interaction of energy with specific chemical species to generate the cascade of information, which are needed for the visual processing. At the end, it sounds natural that the very same physical-chemical interactions, aside from producing a sudden perception of energy provide a trophic activity. In line with this, the photo-pigment melanin is essential to promote the growth of the sensory organ in the inner ear (Ganesan et al., 2008). The autophagy induction seems to be the final common pathway and recent finding indicate that intravitreal gene therapy to produce autophagy activation works in retinal disorders may pave the way to study beneficial effect in the course of acute and chronic disorders where retinal damage is widespread (Liu et al., 2022). In line with this, a disorder of the photopigment melanin leads to both auditory and visual disorders such as (Waardenburg's syndrome) and age related macular degeneration (Ganesan et al., 2008). It is remarkable that, according to gene analysis ninety-two novel genes were identified to support pigment production however secondary validation identified a large panel genes involved in promoting autophagy. This makes a bridge between melanogenesis and autophagy, which represents a proof of concept about the remarkable convergence between things, which were remote at first glance. These astonishing connections bring us back to the use of sound to mimic light in the music by Richard Strauss (Also sprach Zaratustra) where light is represented by pure sounds, which anticipate and takes over light.

Now the natural question which emerges is what was more ancient in the sensory epithelia is the acoustic or visual so we may merge sound and light word and vision by citing the prophet John in his Greek original:

Ἐν ἀρχῇ ἦν ὁ λόγος, καὶ ὁ λόγος ἦν πρὸς τὸν θεόν, καὶ θεὸς ἦν ὁ λόγος. οὗτος ἦν ἐν ἀρχῇ πρὸς τὸν θεόν. πάντα δι᾽ αὐτοῦ ἐγένετο, καὶ χωρὶς αὐτοῦ ἐγένετο οὐδὲ ἕν. ὃ γέγονεν ἐν αὐτῷ ζωὴ ἦν, καὶ ἡ ζωὴ ἦν τὸ φῶς τῶν ἀνθρώπων· καὶ τὸ φῶς ἐν τῇ σκοτίᾳ φαίνει, καὶ ἡ σκοτία αὐτὸ οὐ κατέλαβεν.

At the beginning the Word, the Word was close to God and the Word was God. He was in principle close to God: through him everything was created, and without him nothing now existing was ever made. In him the life, in him the light of the mankind; the light brightness in the shadow but the shadow did not host light.

References

Ambati J. and Fowler B.J. Mechanisms of age-related macular degeneration. *Neuron*, **75**: 26-39, 2012.

Arruri V. and Vemuganti R. Role of autophagy and transcriptome regulation in acute brain injury. *Exp. Neurol.,* **352**: 114032, 2022.

Berry M. Plasticity in the visual system and visually guided behavior. *Adv. Psychobiol.,* **3**: 125-192, 1976.

Biagioni F., Celli R., Giorgi F.S., Nicoletti F., Fornai F. Perspective on mTOR- dependentProtection in Status Epilepticus. *Curr. Neuropharmacol*., Oct **5**.doi: 10.2174/1570159X19666211005152618, 2021.

Bilbao-Malavé V., González-Zamora J., Saenz de Viteri M., de la Puente M., Gándara E., Casablanca-Piñera A., Boquera-Ventosa C., Zarranz-Ventura J., Landecho M.F., García-Layana A. Persistent Retinal Microvascular Impairment in COVID-19 Bilateral Pneumonia at 6-Months Follow-Up Assessed by Optical Coherence Tomography Angiography. *Biomedicines,* **9**: 502, 2021.

Blasiak J., Petrovski G., Veréb Z., Facskó A., Kaarniranta K. Oxidative stress, hypoxia, and autophagy in the neovascular processes of age-related macular degeneration. *Biomed. Res. Int*., **2014**: 768026, 2014.

Bonilha V.L. Age and disease-related structural changes in the retinal pigment epithelium. *Clin. Ophthalmol*., **2**: 413-424, 2008.

Boulton M. and Dayhaw-Barker P. The role of the retinal pigment epithelium: topographical variation and ageing changes. *Eye (Lond),* **15**: 384-389, 2001.

Castino R., Lazzeri G., Lenzi P., Bellio N., Follo C., Ferrucci M., Fornai F., Isidoro C. Suppression of autophagy precipitates neuronal cell death following low doses of methamphetamine. *J. Neurochem*., **106**: 1426-1439, 2008.

Ceccariglia S., Cargnoni A., Silini A.R., Parolini O. Autophagy: a potential key contributor to the therapeutic action of mesenchymal stem cells. *Autophagy*, **16**: 28-37, 2020.

Chang N.C. Autophagy and Stem Cells: Self-Eating for Self-Renewal. *Front. Cell Dev. Biol*., **8**: 138, 2020.

Chen F.K., Agelis L.E., Peh K.K., Teong J., Wong E.N. Factors Contributing to Discrepancy Between Visual Acuity Fractions Derived From a Snellen Chart and Letter Scores on the Early Treatment Diabetic Retinopathy Study Chart. *Asia Pac. J. Ophthalmol. (Phila).*, **3**: 277-285, 2014.

Chen X., Gao F., Lin C., Chen A., Deng J., Chen P., Lin M., Xie B., Liao Y., Gong C., Zheng X. mTOR-mediated autophagy in the hippocampus is involved in perioperative neurocognitive disorders in diabetic rats. *CNS Neurosci. Ther.,* **28**: 540-553, 2022.

Chew E.Y., Sperduto R.D., Hiller R., Nowroozi L., Seigel D., Yanuzzi L.A., Burton T.C., Seddon J.M., Gragoudas E.S., Haller J.A., Blair N.P., Farber M. Clinical course of macular holes: the Eye Disease Case-Control Study. *Arch Ophthalmol*., **11**: 242-246, 1999.

Chiang C.F., Hsu Y.H., Liu C.C., Liang P.C., Miaw S.C., Lin W.L. Pulsed-wave Ultrasound Hyperthermia Enhanced Nanodrug Delivery Combined with Chloroquine Exerts Effective Antitumor Response and Postpones Recurrence. *Sci. Rep*., **9**: 12448, 2019.

Ferrucci M., Pasquali L., Ruggieri S., Paparelli A., Fornai F. Alpha-synucleinand autophagy as common steps in neurodegeneration. *Parkinsonism. Relat. Disord*., **14**: S180-S184, 2008.

Filippone A., Esposito E., Mannino D., Lyssenko N., Praticò D. The contribution of altered neuronal autophagy to neurodegeneration. *Pharmacol.Ther*., **238**: 108178, 2022.

Foglietta F., Canaparo R., Francovich A., Arena F., Civera S., Cravotto G., Frairia R., Serpe L. Sonodynamic treatment as an innovative bimodal anticancer approach: shock wave-mediated tumor growth inhibition in a syngeneic breast cancer model. *Discov. Med*., **20**: 197-205, 2015.

Fornai F., Longone P., Ferrucci M., Lenzi P., Isidoro C., Ruggieri S., Paparelli A. Autophagy and amyotrophic lateral sclerosis: The multiple roles of lithium. *Autophagy*, **4**: 527-530, 2008b.

Fornai F., Longone P., Cafaro L., Kastsiuchenka O., Ferrucci M., Manca M.L., Lazzeri G., Spalloni A., Bellio N., Lenzi P., Modugno N., Siciliano G., Isidoro C., Murri L., Ruggieri S., Paparelli A. Lithiumdelaysprogression of amyotrophiclateralsclerosis. *Proc. Natl. Acad. Sci. U S A*, **105**: 2052-2057, 2008a.

Freedman M.S., Lucas R.J., Soni B., von Schantz M., Munoz M., David-Gray Z., Foster R., Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science*, **284**: 502-504, 1999.

Ganesan A.K., Ho H., Bodemann B., Petersen S., Aruri J., Koshy S., Richardson Z., LeL.Q., Krasieva T., Roth M.G., Farmer P., White M.A. Genome-wide siRNA-based functional genomics of pigmentation identifies novel genes and pathways that impact melanogenesis in human cells. *PLoS Genet*., **4**: e1000298, 2008.

Gass J.D. Drusen and disciform macular detachment and degeneration. Trans.*Am. Ophthalmol. Soc*., **70**: 409-436, 1972.

Goel N. and Shukla G. Long-term follow up of en face optical coherence tomography of the inner retinal surface following internal limiting membrane peeling for idiopathic macular holes. *Int. Ophthalmol*., **41**: 1003-1010, 2021.

Guo T., Liu T., Sun Y., Liu X., Xiong R., Li H., Li Z., Zhang Z., Tian Z., Tian Y. Sonodynamic therapy inhibits palmitate-induced beta cell dysfunction via PINK1/Parkin-dependent mitophagy. *Cell Death Dis*., **10**: 457, 2019.

Huang X., Niu L., Meng L., Lin Z., Zhou W., Liu X., Huang J., Abbott D., Zheng H. Transcranial Low-Intensity Pulsed Ultrasound Stimulation Induces Neuronal Autophagy. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*., **68**: 46-53, 2021.

Hwang I., Tang D., Paik J. Oxidative stress sensing and response in neural stem cell fate. *Free Radic. Biol. Med.,* **169**: 74-83, 2021.

Intartaglia D., Giamundo G., Conte I. Autophagy in the retinal pigment epithelium: a new vision and future challenges. *FEBS J*., **16**. doi: 10.1111/febs.16018, 2021.

Isidoro C., Biagioni F., Giorgi F.S., Fulceri F., Paparelli A., Fornai F. The role of autophagy on the survival of dopamine neurons. *Curr. Top. Med. Chem*., **9**: 869-879, 2009.

Johnson A.T., Dooly C.R., Simpson C.R. Generating the Snellen Chart by computer. *Comput. Methods Programs Biomed.*, **57**: 161-166, 1998.

Kaarniranta K., Blasiak J., Liton P., Boulton M., Klionsky D.J., Sinha D. Autophagy in age-related macular degeneration. *Autophagy*, **1**: 1-13, 2022.

Kaarniranta K., Uusitalo H., Blasiak J., Felszeghy S., Kannan R., Kauppinen A., Salminen A., Sinha D., Ferrington D. Mechanisms of mitochondrial dysfunction and their impact on age-related macular degeneration. *Prog. Retin. Eye Res*., **79**: 100858, 2020.

Kanno H., Handa K., Murakami T., Aizawa T., Ozawa H. Chaperone-Mediated Autophagy in Neurodegenerative Diseases and Acute Neurological Insults in the Central Nervous System. *Cells*, **11**: 1205, 2022.

Kim K.L., Joo K., Park S.J., Park K.H., Woo S.J. Progression from intermediate to neovascular age related macular degeneration according to drusen subtypes: Bundang AMD cohort study report 3. *ActaOphthalmol*., **100**: e710-e718, 2021.

Kim J.Y., Kim R.Y., Kim M., Park Y.G., Park Y.H. Progression rate analysis of idiopathic macular hole using optical coherence tomography before vitrectomy: short-term results. *ActaOphthalmol*., Mar **25**, 2022.

Kozlowski M.R. RPE cell senescence: a key contributor to age-related macular degeneration. *Med. Hypotheses,* **78**: 505-510, 2012.

Li Y., Sun C., Feng G., He Y., Li J., Song J. Low-intensity pulsed ultrasound activates autophagy in periodontal ligament cells in the presence or absence of lipopolysaccharide. *Arch. Oral. Biol*., **117**: 104769, 2020.

Liu J., Bassal M., Schlichting S., Braren I., Di Spiezio A., Saftig P., Bartsch U.Intravitreal gene therapy restores the autophagy-lysosomal pathway andattenuates retinal degeneration in cathepsin D-deficient mice. *Neurobiol. Dis*., **164**: 105628, 2022.

Lucas R.J., Douglas R.H., Foster R.G., Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat. Neurosci*., **4**: 621e626, 2001.

Madeo F., Eisenberg T., Kroemer G. Autophagy for the avoidance of neurodegeneration. *Genes Dev*., **23**: 2253-2259, 2009.

Maiti P., Peruzzaro S., Kolli N., Andrews M., Al-Gharaibeh A., Rossignol J., Dunbar G.L. Transplantation of mesenchymal stem cells overexpressing interleukin-10 induces autophagy response and promotes neuroprotection in a rat model of TBI. *J.Cell Mol. Med*., **23**: 5211-5224, 2019. Erratum in: *J. Cell Mol. Med*., **26**: 241, 2022.

Mansour H.A., Uwaydat S.H., Parodi M., Jürgens I., Smiddy W., Allabban A.A., Schwartz S.G., Foster R.E., Ascaso J., Leoz M.S., Belotto S., Mateo J., Olivier-Pascual N., Lima L.H., Navea A., Neila E.M.R., Castillo R.A., Alaman A.S., Mansour A.M. Collaborators of the Nonsurgical Resolution of Macular Hole Study Group. Recovery course of foveal microstructure in the nonsurgical resolution of full-thickness macular hole. *Graefes. Arch.Clin. Exp. Ophthalmol.*, Apr **25**, 2022.

McCarty M.F. Nutraceutical and Dietary Strategies for Up-Regulating Macroautophagy. *Int. J. Mol. Sci.*, **23**: 2054, 2022.

Movahedpour A., Vakili O., Khalifeh M., Mousavi P., Mahmoodzadeh A., Taheri-Anganeh M., Razmeh S., Shabaninejad Z., Yousefi F., Behrouj H., Ghasemi H., Khatami S.H. Mammalian target of rapamycin (mTOR) signaling pathway and traumatic brain injury: A novel insight into targeted therapy. *Cell Biochem. Funct.*, **40**: 232-247, 2022.

Nita M. and Grzybowski A. Interplay between reactive oxygen species and autophagy in the course of age-related macular degeneration. *EXCLI J.*, **19**: 1353-1371, 2020.

Pevna V., Wagnières G., Huntosova V. Autophagy and Apoptosis Induced in U87 MGGlioblastoma Cells by Hypericin-Mediated Photodynamic Therapy Can Be Photobiomodulated with 808 nm Light. *Biomedicines*, **9**: 1703, 2021.

Pinelli R., Bertelli M., Scaffidi E. The first clinical case of dry age-related macular degeneration treated with photobiomodulation and nutraceuticals: a protocol proposal. *CellR4*, **8**: e2833, 2020c.

Pinelli R., Bertelli M., Scaffidi E., Bumah V.V., Biagioni F., Busceti C.L., Puglisi-Allegra S., Fornai F. The neurobiology of nutraceuticals combined with light exposure, a case report in the course of retinal degeneration. *Arch. Ital. Biol.*, **159**: 134-150, 2021b.

Pinelli R., Bertelli M., Scaffidi E., Busceti C.L., Biagioni F., Fornai F. Exosomes and alpha-synuclein within retina from autophagy to protein spreading in neurodegeneration. *Arch. Ital. Biol.*, **159**: 38-50, 2021a.

Pinelli R., Biagioni F., Bertelli M., Busceti C.L., Scaffidi E., Ryskalin L., Fornai F. Retinal Degeneration Following Chronic Administration of the Parkinsonism-Inducing Neurotoxin MPTP. *Arch. Ital. Biol.*, **159**: 64-81, 2021c.

Pinelli R., Bertelli M., Scaffidi E., Fulceri F., Busceti C.L., Biagioni F., Fornai F. Measurement of drusen and their correlation with visual symptoms in patients affected by age-related macular degeneration. *Arch. Ital. Biol.*, **158**: 82-104, 2020d.

Pinelli R., Bertelli M., Scaffidi E., Polzella M., Fulceri F., Biagioni F., Fornai F. Nutraceuticals for dry age-related macular degeneration: a case report based on novel pathogenic and morphological insights. *Arch. Ital. Biol.*, **58**: 24-34, 2020b.

Pinelli R., Biagioni F., Limanaqi F., Bertelli M., Scaffidi E., Polzella M., Busceti C.L., Fornai F. A Re-Appraisal of Pathogenic Mechanisms Bridging Wet and Dry Age-Related Macular Degeneration Leads to Reconsider a Role for Phytochemicals. *Int. J. Mol. Sci.*, **21**: 5563, 2020a.

Pinelli R., Bucci D., Scaffidi E., Berti C., Bumah V., Lazzeri G., Ruffoli R., Puglisi-Allegra S., Busceti C.L., Fornai F. Noradrenergic substrates sensing light within brainstem reticular formation as targets for light-induced behavioral and cardiovascular plasticity. *Arch. Ital. Biol,* In press, 2022.

Ravikumar B. and Rubinsztein D.C. Can autophagy protect against neurodegeneration caused by aggregate-prone proteins? *Neuroreport,* **15**: 2443-2445, 2004.

Recanzone G.H. Interactions of auditory and visual stimuli in space and time. *Hear Res.*, **258**: 89-99, 2009.

Ricciardi E., Bonino D., Sani L., Vecchi T., Guazzelli M., Haxby J.V., Fadiga L., Pietrini P. Do we really need vision? How blind people "see" the actions of others. *J. Neurosci.*, **29**: 9719-9724, 2009.

Rose S.P. Early visual experience, learning, and neurochemical plasticity in the rat and the chick. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **278**: 307-318, 1977.

Rubinsztein D.C., DiFiglia M., Heintz N., Nixon R.A., Qin Z.H., Ravikumar B., Stefanis L., Tolkovsky A. Autophagy and its possible roles in nervous system diseases, damage and repair. *Autophagy*, **1**: 11-22, 2005.

Sethna S., Scott P.A., Giese A.P.J., Duncan T., Jian X., Riazuddin S., Randazzo P.A., Redmond T.M., Bernstein S.L., Riazuddin S., Ahmed Z.M. CIB2 regulates mTORC1 signaling and is essential for autophagy and visual function. *Nat. Commun.*, **12**: 3906, 2021.

Shijo T., Sakurada Y., Tanaka K., Miki A., Yoneyama S., Machida Y., Chubachi A., Wakatsuki Y., Sugiyama A., Onoe H., Kikushima W., Mori R., Kashiwagi K. Drusenoid Pigment Epithelial Detachment: Genetic and Clinical Characteristics. *Int. J. Mol. Sci.*, **22**: 4074, 2021.

Stefenon L., Boasquevisque M., Garcez A.S., de Araújo V.C., Soares A.B., Santos-Silva A.R., Sperandio F., Brod J.M.M., Sperandio M. Autophagy upregulation may explain inhibition of oral carcinoma in situ by photobiomodulation in vitro. *J. Photochem. Photobiol. B.*, **221**: 112245, 2021.

Stino H., Wassermann L., Ristl R., Abela-Formanek C., Georgopoulos M., Sacu S., Schmidt-Erfurth U., Pollreisz A. Evaluation of neuroretinal integrity in optical coherence tomography-graded eyes with partial-thickness macular holes. *ActaOphthalmol.,* Apr **11**, 2022.

Strettoi E., Di Marco B., Orsini N., Napoli D. RetinalPlasticity. *Int. J. Mol. Sci.*, **23**: 1138, 2022.

Su D., Greenberg A., Simonson J.L., Teng C.C., Liebmann J.M., Ritch R., Park S.C. Efficacy of the Amsler Grid Test in Evaluating Glaucomatous Central Visual Field Defects. *Ophthalmology*, **123**: 737-743, 2016.

Suárez-Barrio C., Del Olmo-Aguado S., García-Pérez E., Fernández-Vega-Cueto L., Fernández-Vega Cueto A., Baamonde-Arbaiza B., Fernández-Vega L., Merayo-Lloves J. Plasma Rich in Growth Factors Promotes Autophagy in ARPE19 Cells in Response to Oxidative Stress Induced by Blue Light. *Biomolecules*, **11**: 954, 2021.

Tonti E., Budini M., Vingolo E.M. Visuo-Acoustic Stimulation's Role in Synaptic Plasticity: A Review of the Literature. *Int. J. Mol. Sci.*, **22**: 10783, 2021.

Usategui-Martín R., Puertas-Neyra K., Galindo-Cabello N., Hernández-Rodríguez L.A., González-Pérez F., Rodríguez-Cabello J.C., González-Sarmiento R., Pastor J.C., Fernandez-Bueno I. Retinal Neuroprotective Effect of Mesenchymal Stem Cells Secretome Through Modulation of Oxidative Stress, Autophagy, and Programmed Cell Death. *Invest. Ophthalmol. Vis. Sci.*, **63**: 27, 2022.

Vogt D., Haritoglou C., Mautone L., Hagenau F., Guenther S.R., Wolf A., Priglinger S.G., Schumann R.G. Premacular Cells as Source of Neurotrophic Factors in Idiopathic Macular Holes. *Curr. Eye Res.*, **45**: 1395-1402, 2020.

Wang Y., Liu X., Zhang W., He S., Zhang Y., Orgah J., Wang Y., Zhu Y. Synergy of "Yiqi" and "Huoxue" components of QishenYiqi formula in ischemic stroke protection via lysosomal/inflammatory mechanisms. *J. Ethnopharmacol.,* **293**: 115301, 2022a.

Wang F., Xia Z., Sheng P., Ren Y., Liu J., Ding L., Yan B.C. Targeting the Erk1/2 and autophagy signaling easily improved the neurobalst differentiation and cognitive function after young transient forebrain ischemia compared to old gerbils. *Cell Death Discov.*, **8**: 87, 2022b.

Wang X., Lin Q., Zhang T., Wang X., Cheng K., Gao M., Xia P., Li X. Low-intensity pulsed ultrasound promotes chondrogenesis of mesenchymal stem cells via regulation of autophagy. *Stem Cell Res. Ther.*, **10**: 41, 2019.

Wu Z., Fletcher E.L., Kumar H., Greferath U., Guymer R.H. Reticular pseudodrusen: A critical phenotype in age-related macular degeneration. *Prog. Retin. Eye Res.*, **6**: 101017, 2021.

Xia P, Wang Q, Song J, Wang X, Wang X, Lin Q, Cheng K, Chen A, Li X. Low-Intensity Pulsed Ultrasound Enhances the Efficacy of Bone Marrow-Derived MSCs in Osteoarthritis Cartilage Repair by Regulating Autophagy-Mediated Exosome Release. *Cartilage*, **13**: 19476035221093060, 2022.

Xia P., Wang X., Wang Q., Wang X., Lin Q., Cheng K., Li X. Low-Intensity Pulsed Ultrasound Promotes Autophagy-Mediated Migration of Mesenchymal Stem Cells and Cartilage Repair. *Cell Transplant.*, **30**: 963689720986142, 2021.

Xiao D., Lv J., Zheng Z., Liu Y., Zhang Y., Luo C., Qi L., Qin B., Liu C. Mechanisms of microRNA-142 in mitochondrial autophagy and hippocampal damage in a rat model of epilepsy. *Int. J. Mol. Med.,* **47**: 98, 2021.

Xu Y., Liu Z., Xu S., Li C., Li M., Cao S., Sun Y., Dai H., Guo Y., Chen X., Liang W. Scientific Evidences of Calorie Restriction and Intermittent Fasting for Neuroprotection in Traumatic Brain Injury Animal Models: A Review of the Literature. *Nutrients*, **14**: 1431, 2022.

Xu Z., Tian N., Li S., Li K., Guo H., Zhang H., Jin H., An M., Yu X. Extracellular vesicles secreted from mesenchymal stem cells exert anti-apoptotic and anti-inflammatory effects via transmitting microRNA-18b in rats with diabetic retinopathy. *Int.Immunopharmacol.*, **101**: 108234, 2021.

Yako T., Nakamura M., Nakamura S., Hara H., Shimazawa M. Pharmacological inhibition of mitochondrial fission attenuates oxidative stress-induced damage of retinal pigmented epithelial cells. *J. Pharmacol. Sci.*, **146**: 149-159, 2021.

Yang K.L., Khoo B.Y., Ong M.T., Yoong I.C.K, Sreeramanan S. In vitro anti-breast cancer studies of LED red light therapy through autophagy. *Breast Cancer*, **28**: 60-66, 2021.

Zeng Q., Hong S., Wang X., Cheng Y., Sun J., Xia W. Regulation of exosomes secretion by low-intensity pulsed ultrasound in lung cancer cells. *Exp. Cell Res.*, **383**: 111448, 2019.

Zhang L., Dai L., Li D. Mitophagy in neurological disorders. *J. Neuroinflammation.,* **18**: 297, 2021.

Zhang L., Li Z., Mao L., Wang H. Circular RNA in Acute Central Nervous System Injuries: A New Target for Therapeutic Intervention. *Front. Mol. Neurosci.*, **15**: 816182, 2022.

Zhou H.Y., Li Q., Wang J.X., Xie Y.J., Wang S.Q., Lei L., Gao Y.Q., Huang M.M., Hu Y., Xu F.Y., Zhang C. Low-intensity pulsed ultrasound repair in mandibular condylar cartilage injury rabbit model. *Arch. Oral. Biol.*, **104**: 60-66, 2019.

Zilber N., Ciuciu P., Gramfort A., Azizi L., van Wassenhove V. Supramodal processing optimizes visual perceptual learning and plasticity. *Neuroimage*, **93**: 32-46, 2014.