Spontaneous and visible light-induced ultraweak photon emission from rat eyes

Chao Wang[1], **István Bókkon**[2*], **Jiapei Dai**[1], **István Antal**[3]

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[1]Wuhan Institute for Neuroscience and Neuroengineering, South-Central University for Nationalities, Wuhan, China

[2]Doctoral School of Pharmaceutical and Pharmacological Sciences, Semmelweis University, Budapest, Hungary

[3]Department of Pharmaceutics, Semmelweis University, Budapest, Hungary

Corresponding author email: bokkoni@yahoo.com

Co-Corresponding author email: jdai@mail.scuec.edu.cn

Abstract

Here, we present the first experimental *in vitro* evidence of the existence of spontaneous and visible light induced ultraweak photon emission from freshly isolated whole eye, lens, vitreous humor and retina samples from rats. These results suggest that the photochemical source of retinal discrete noise, as well as retinal phosphenes, may originate from natural bioluminescent photons within the eyes. During normal vision, the eyes are continuously exposed to ambient powerful photons that pass through various parts of the eyes, which can produce ultraweak delayed bioluminescent photons that arise from diverse parts of the eyes. Although the importance and possible role of ambient light induced permanent delayed photons (within different parts of the eyes) during vision requires further investigation, our study may provide evidence of an origin of discrete dark noise and retinal phosphenes.

Key words: bioluminescent photons, lipid peroxidation, discrete dark noise, retinal phosphenes

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1. Introduction

During natural metabolic processes, continuous and spontaneous ultraweak photon emissions (UPEs) have been observed without any excitation from any kind of living organisms or cells (Quickenden and Que Hee, 1974; Tilbury and Cluickenden, 1988; Scott et al., 1991; Devaraj et al., 1991; Takeda et al., 1998; Kobayashi et al., 1999a; Kobayashi and Inaba, 2000; Niggli et al., 2001; Takeda et al., 2004; Nakano, 2005; Mansfield, 2005; Yoon et al., 2005; Van Wijk et al., 2006; Laager et al., 2008; Tafur et al., 2010). UPEs of biological systems are very weak electromagnetic waves in the optical range of the spectrum. These spontaneous photon emissions are known by many names including biophotons, low-intensity chemiluminescence, dark luminescence, ultraweak electromagnetic light, ultraweak bioluminescence, ultraweak autoluminescence and ultraweak photons. UPEs cannot be seen by the naked eye but can be measured by very sensitive instruments, such as a photomultiplier tube (PMT) or an electron multiplier CCD (EM-CCD) camera, as well as by in situ biophoton autography (Sun et al., 2010).

UPEs are attributable to the endogenous production of diverse biochemical reactions, especially bioluminescent radical reactions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their derivates, as well as the simple cessation of excited states during natural oxidative processes. There are several possible sources of UPEs within cells including mitochondrial respiration chains, lipid peroxidation, peroxisomal reactions, oxidation of catecholamines, and oxidation of tyrosine and tryptophan residues in proteins (Kruk et al., 1989; Watts et al., 1995; Nakamo, 2005; Catalá, 2006). These ultraweak photon emissions are comprised of various ranges of wavelengths including infrared, visible, and ultraviolet ranges. UPEs originate mainly from mitochondrial oxidative metabolism and lipid peroxidation that produce light-emitting molecules such as triplet carbonyls and singlet oxygen (Nakamo, 2005; Thar and Kühl, 2004).

Neural cells also generate constantly ultraweak photons during their natural metabolism (Isojima et al., 1995; Kobayashi et al., 1999a; Kobayashi et al., 1999b). *In vivo* UPEs from rat brains are associated with electroencephalogram (EEG) activity, cerebral energy metabolism, and oxidative processes (Kobayashi et al., 1999b). UPEs from neural tissue depend on the membrane depolarization and Ca^{2+} entry into cells (Kataoka et al., 2001). Neural activity-dependent UPEs have been demonstrated in the rat hippocampus (Kataoka et al., 2001). Recently, additional evidence by Sun et al. (2010) revealed that ultraweak bioluminescent photons can conduct along the neural fibers and can be considered

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a means of neural communication. They have also been suggested that biophotonic and bioelectronic activities are not independent biological events in the nervous system, and their synergic action may play an important role in neural signal transductions.

Recently, Bókkon (2008) proposed a novel biophysical (*redox molecular*) interpretation of phosphene lights. He argued that phosphenes could arise from unregulated overproduction of free radicals and excited biomolecules in various parts of the visual system (Bókkon, 2008). Unregulated overproduction of free radicals and excited species can generate a brief increase of UPEs in different regions of the visual system, and if this excess of UPEs exceeds a threshold, they can appear as phosphene lights in our mind. Among those phosphenes which share this common feature are electrical, magnetic, ionizing radiation, and mechanical-induced phosphenes, as well as phosphenes arising from exposure to various drugs, stress, and optic nerve diseases (Bókkon, 2008). In addition, Bókkon and Vimal (2009) suggested that the discrete dark noise of rods (*in dark-adapted retinal cells*) may result from the bioluminescent photons generated continuously by retinal lipid peroxidation and oxidative metabolism.

According to electrical recordings, rods have two components of the dark noise: a constant, low amplitude component (*amplitude » 0.2 pA*) and a discrete component (*amplitude » 1 pA*) (Schwartz, 1977; Baylor et al., 1980). The continuous component of a rod's noise originates from the spontaneous activation of cGMP phosphodiesterase molecules (Rieke and Baylor, 1996). Spontaneous activation of rhodopsin produces discrete dark noises indistinguishable from single-photon responses (Baylor et al., 1980). In a retinal photoreceptor cell, a visual pigment molecule can be activated not only by absorption of a photon but also by thermal energy. Current estimates of the activation energies for these two processes in vertebrate rod and cone pigments are on the order of 40 – 50 kcal / mol for activation by light and 20 – 25 kcal / mol for activation by heat (Ala-Laurila et al., 2004).

To resolve this discrepancy, it was suggested that photon and thermal activation of rhodopsin follow different biochemical processes. The most persuasive argument for a separate low-energy thermal pathway stems from the observation that the discrete noises in rods occur in a small subpopulation of rhodopsins, where the Schiff base linking the chromophore to the protein part has been deprotonated (Barlow et al., 1993). According to this hypothesis, the dark event rate should be strongly dependent on pH. However, observations indicating that thermal pigment activation depends on prior deprotonation of the Schiff base contradict this hypothesis (Firsov et al., 2002). Thus, new hypotheses have been developed to account for this contradiction. Ala-Laurila et al. (2004) suggested that the low-

energy thermal activation pathway may not exist and is simply an analytical artifact. According to this hypothesis, activation by heat and by light may follow the same molecular route. Lorenz-Fonfria et al. (2010) proposed that thermodynamic and kinetic structural fluctuations of protein may facilitate retinal thermal isomerization of rhodopsin. However, a molecular theory that can present an adequate explanation for the discrete retinal dark events of vertebrate rhodopsin remains undeveloped.

Since natural lipid peroxidation is one of the main sources of UPEs (Nakano, 2005; Adam et al., 2005; Thar and Kühl, 2004; Catalá, 2006), and photoreceptors have the highest oxygen consumption and polyunsaturated fatty acid concentration in the body (Fliesler and Anderson, 1983), permanent UPEs should occur in the retina without any external photonic stimulation. Thus, Bókkon and Vimal's (2009) photochemical (*biophysical*) interpretation about the discrete dark noise of rods may be a possible explanation for the discrete retinal dark. However, to date, experimental evidence of spontaneous or visible light induced ultraweak photon emissions in human or animal eyes has not been reported, but it is necessary to support the hypotheses set forth by Bókkon (2008) and colleagues (Bókkon and Vimal, 2009). Here, we present the first *in vitro* evidence of spontaneous and visible light induced UPEs from freshly isolated whole eye, lens, vitreous humor and retina samples from rats.

2. Results

All isolated samples (*whole eye, lens, vitreous humor and retina*) presented continuous, spontaneous and basal photon emission without any excitation. In addition, after 300 sec of basal recording followed by 30 min of adaptation to the dark, isolated rat whole eye, lens, vitreous humor and retina were illuminated by monochromatic red, green or blue light with 10, 20 or 40 sec duration, respectively. As shown in Figure 1, all samples presented continuous spontaneous biophoton emission and obvious delayed biophoton emission (Fig. 1 A-C; E-G; I-K; M-O). All figures (Fig. 1 A-C; E-G; I-K; M-O) show a similar and typical curve with an initial tiny peak following a quick decrease, and delayed biophoton emission reaches a baseline level within 10 min. The effects of delayed biophoton emission were affected by different spectrum light and illumination time and also depended on the different parts of the eye (Fig. 1 A-C; E-G; I-K; M-O). We compared the peak values in different spectrum light illumination using three different illumination times (10, 20 or 40 sec) for the whole eye and retina. The results of this analysis showed a significantly larger peak value in 20 or 40 sec blue light illumination than in 10 sec blue light illumination (Fig. 1D and H).

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For green light illumination, differences were observed between 40 and 10 sec, but not 20 and 10 sec for all samples (see Fig. 1D, H, L and P). No significant differences were found between 40, 20 and 10 sec after red light illumination for the retina, lens and vitreous humor with the exception of whole eye (Fig. 1D, H, L and P). The blue light illumination appeared to show some differences for the lens and vitreous humor as compared to the whole eye and retina (Fig. 1D, H, L and P), though this may be due to the difference of tissue characteristics between lens, vitreous humor, retina and whole eye. In summary, the current results showed that 40 sec exposure to red, green or blue light illumination caused delayed biophoton emission to reach peak maximum values. In addition, the effects of red light illumination on biophoton emission were less than those of the green and blue light illuminations (see Fig. 2A-D).

Then we analyzed the differences during the time course of attenuation of the delayed biophoton emission after 40 sec red, green or blue light illumination by comparing the lens, vitreous humor or retina with the whole eye. We found that there existed obvious differences between lens or vitreous humor and whole eye at the most of time points during the course of attenuation of the delayed biophoton emission, but no significant difference between retina and whole eye (Fig. 2E-G).
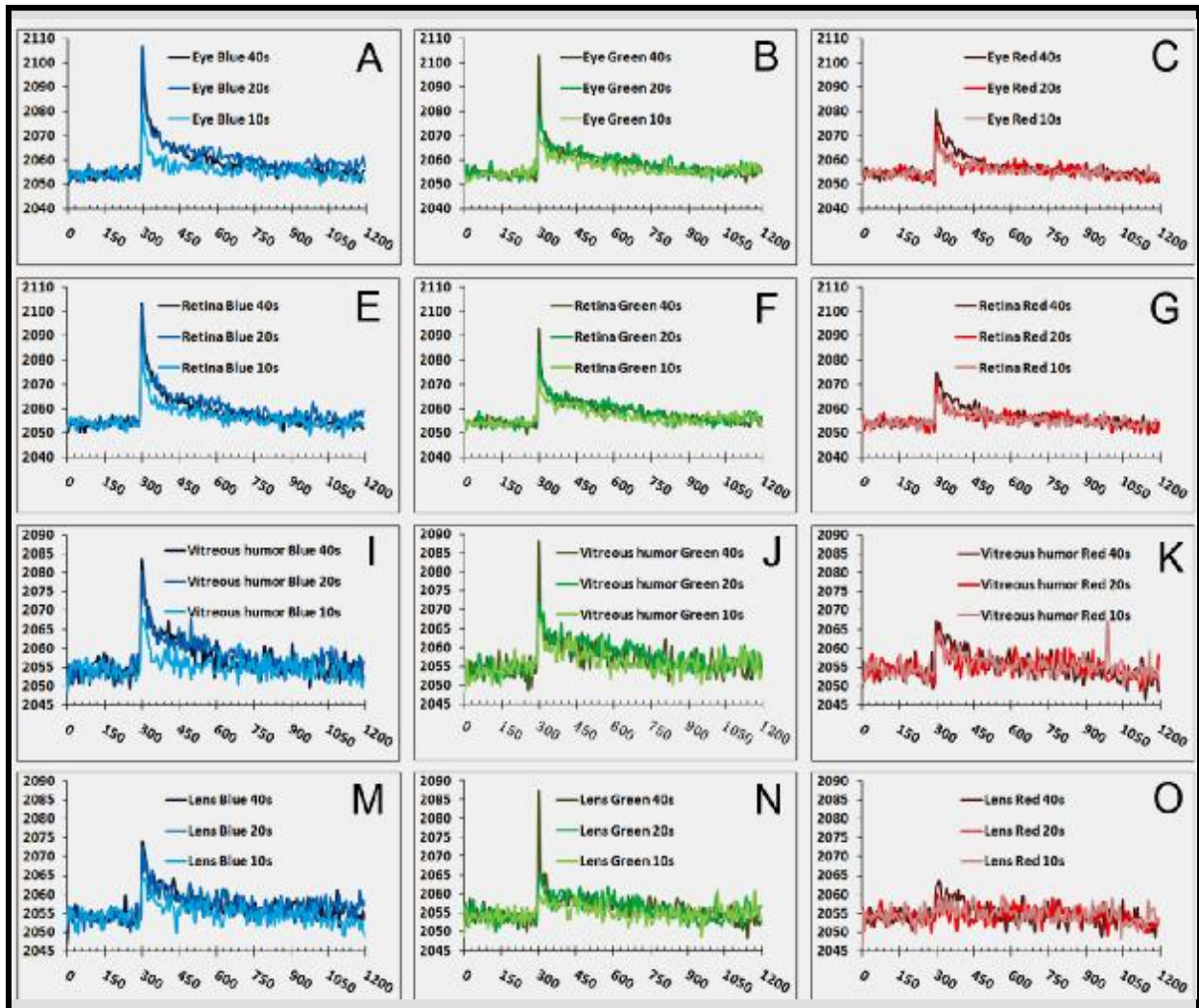
Fig. 1. The time course of attenuation of the delayed biophoton emission after different durations of exposure (10, 20, or 40 sec) to different light illuminations (blue, green or red) on the whole eye, retina, vitreous humor and lens. (A-C; E-G; I-K; M-O): After 300 sec of basal recording (*corresponding to the spontaneous biophoton emission*), all samples showed a similar and typical curve of light induced delayed biophoton emission, with an initial tiny peak following a quick decrease. Baseline levels were reached within 10 min. X axial represents the time course (sec) and real-time imaging by automatically taking an image every 6 sec. Y axial represents the mean value of gray in the image from samples (N = 7). **(D, H, L and P):** The comparison of peak value between different illumination times (10, 20, or 40 sec) after different spectrum light illumination (blue, green or red) on different samples (whole eye, retina, vitreous humor or lens). *P < 0.05, **P < 0.01.
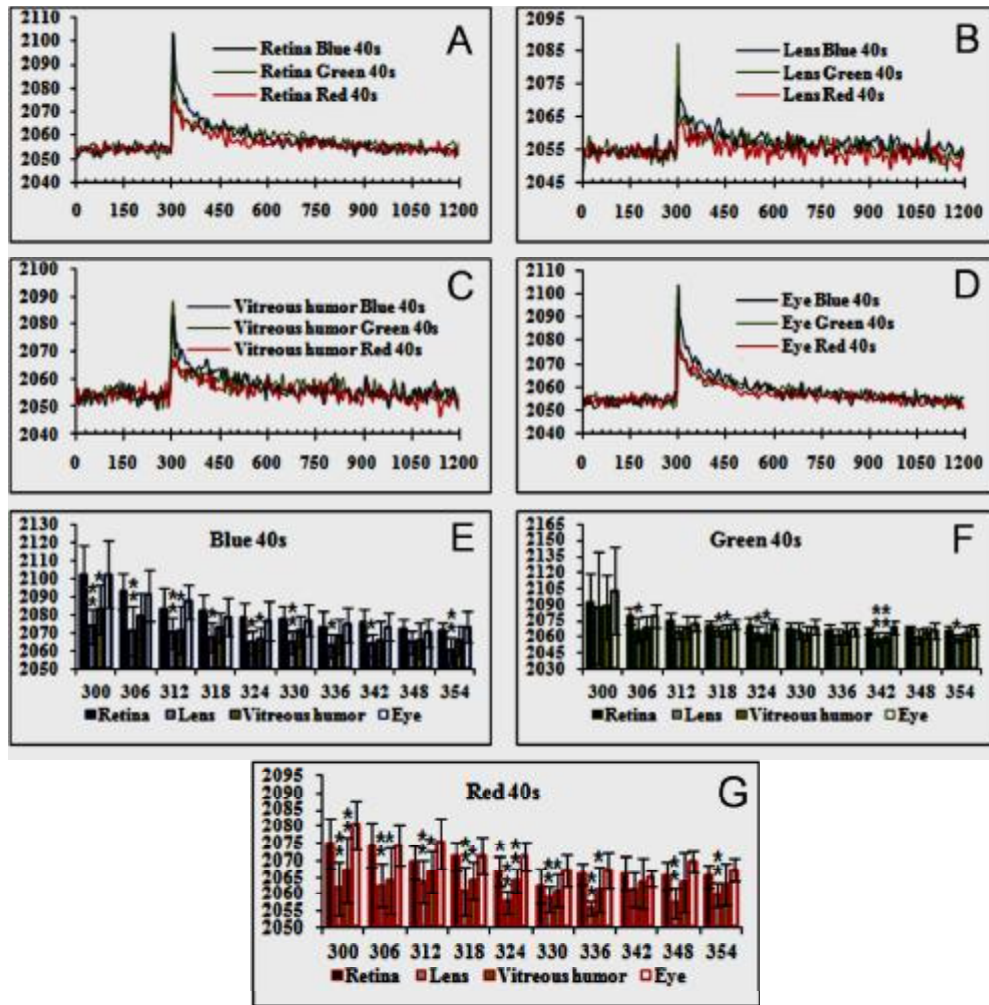
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Fig. 2. The comparison of the time course of attenuation of the delayed biophoton emission after a 40-sec exposure to different light illuminations (blue, green or red) on the whole eye, retina, vitreous humor and lens. (A-D): The effect of red light illumination on the biophoton emission is less than that of green and blue light illumination. X axial represents the time course (sec) and real-time imaging by automatically taking an image every 6 sec. Y axial represents the mean value of gray in the image from samples (N = 7). **(E-G):** Differences between the retina (or vitreous humor or lens) and the whole eye at the different time points during the course of attenuation of the delayed biophoton emission after 40 sec exposure to red, green or blue light illumination (N=7). *P < 0.05, **P < 0.01.

3. Discussion

Dark retinal noise by bioluminescent photons

The present results unambiguously demonstrated that various parts of *in vitro,* freshly isolated rat eye, including the lens, vitreous humor and retina as well as the whole eye, can display continuous ultraweak photon emission. As shown in Figs. 1 and 2, the lens, vitreous humor, retina and the whole eye displayed basic intensities of continuous UPEs. Together, these results indicate that there are several natural sources of UPEs within the eye. During photopic or scotopic vision, continuous UPEs can be negligible. In contrast, in dark-adapted retina, such UPEs, are significant.

Natural lipid peroxidation is one of the major sources of UPEs. Photoreceptors have the highest oxygen consumption and polyunsaturated fatty acid concentration in the body. In addition, reactive oxygen species are constantly formed during cellular metabolism in the retina and are removed by antioxidant defenses. Under regulated circumstances, lipid peroxidation is a natural process in different cells and in retinal membranes (Catalá, 2006), and during normal retinal functioning, external lights produce lipid peroxidation (Dzhafarov et al., 1987). Moreover, Sun et al. (2006) have suggested that oxidative modification of the photoreceptors' outer segment takes place in the retina and that phospholipid peroxidation products act as signaling molecules for retinal pigment epithelium (RPE) phagocytosis.

In addition, the rod (and cone) outer segment membranes are primarily lipoprotein complexes, and rhodopsins (*chromoproteins*) are surrounded by highly enriched polyunsaturated phospholipids. This complex structure allows rhodopsin to easily catch bioluminescent photons originated from surrounding lipid peroxidation. However, a rod cell in the eye can perceive and transform a single photon (*the smallest unit of energy*) of light into a neural signal (Baylor et al., 1979).

We should mention further facts. First, the increased frequency of dark events in photoreceptors exposed to higher temperatures is evidence for the thermal contribution to the generation of dark noise (Baylor et al., 1980). However, free radical formation, lipid peroxidation, and bioluminescent photon emission are also temperature-dependent processes (Player and. Hultin,1977; Alvarez and Storey, 1985; Misík et al., 1994; Lin et al., 2003; Niggli, 2003). Second, dark events are the result of Poisson fluctuations in photon absorption (Field et al., 2005). However, bioluminescent biophoton emissions also bear non-linear, Poisson-like distributions (Kobayashi et al., 1998; Kobayashi and Inaba, 2000; Abele et al., 2002; Popp and Belousov, 2003).

The results of previous studies in addition to our observation that several parts within the eye can perform continuous UPEs may support the bioluminescent interpretation of discrete dark noise of rods, as suggested by Bókkon and Vimal (2009) (see Fig.3). It is also possible that both thermal activation processes and bioluminescent photons simultaneously contribute to the production of discrete dark noises, albeit to different extents. In addition, it may also occur if a bioluminescent photon (*by oxidative reactions*) has lower energy (*to bleach or excite pigment*) than the activation energy of a visual pigment, the thermal energy of the molecule may be large enough to supply the deficit (Srebro, 1966).

Previous reports have shown that cones show a 1000-fold higher level of discrete noise. However, it is highly improbable that cones would be 1000-fold more likely to absorb bioluminescent photons than rods. It seems most likely that discrete dark noise in the primate cones derives not from spontaneous visual pigment isomerization but from constitutive activity in the downstream phototransduction processes, such as the phosphodiesterase (Holcman and Korenbrot, 2005; Fu et al. 2008).
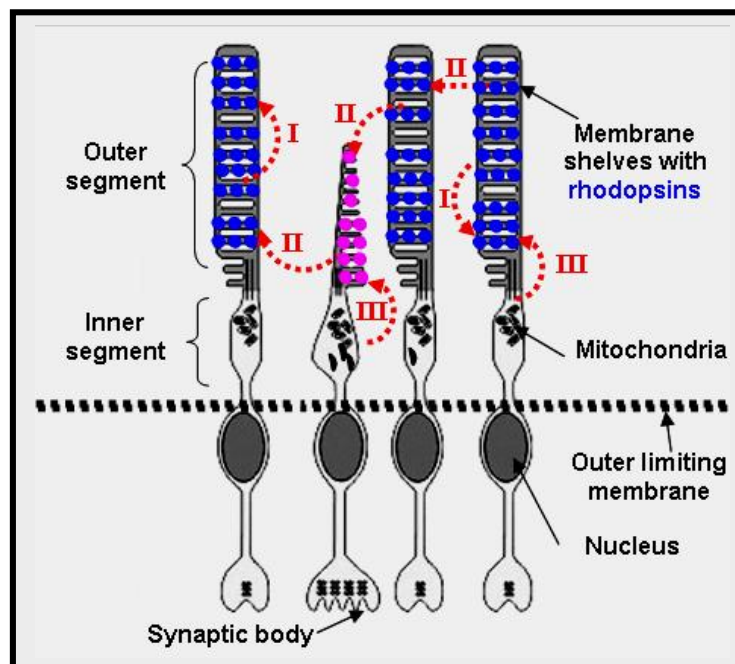

Fig. 3. Discrete dark event in rods and cones by bioluminescent photons as indicated by red curved arrows. **I**. It is possible that a given rod or cone emits a bioluminescent photon that changes its direction, and a subsequently can absorb its own bioluminescent photon. **II.** A rod or cone can absorb bioluminescent photons from the lipid peroxidation of PUFA of adjacent rods or cones. **III.** Bioluminescent photons, emitted from the mitochondrial oxidative metabolism in the inner segment, can also be absorbed by opsins.

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Retinal phosphenes by bioluminescent photons

Phosphene perception is an entoptic phenomenon characterized by the experience of seeing light without light actually entering into the eye (*i.e., without external photons*). Phosphenes can be points, spots, bars or chaotic structures of colored or colorless light. Phosphenes can be elicited by a variety of stimuli (electrical, magnetic, mechanical, etc.) that excite cells in the visual pathway, as well as by random firing of cells in the visual system (Reznikov, 1981; Lindenblatt and Silny, 2002; Merabet et al., 2003). Phosphenes can appear in a variety of diseases of the retina or of the visual pathways, though healthy persons can perceive them as well (Onofrj et al., 1998; Brigatti and Maguluri, 2005). Phosphene generation by electrical stimulation of the visual pathway with implanted electrodes is regarded as a hopeful method for reversing blindness (Dobelle et al., 1974; Zrenner, 2002). Different types of visual implants (*also called visual prostheses*) are named according to their locations: subretinal, epiretinal, optic nerve, lateral geniculate nucleus and cortical (Cohen, 2007).

Vision researchers think that phosphenes result from the electrical activity in various parts of the visual system. A historic and discredited assumption is that phosphene lights are generated within the eye (Grüsser and Hagner, 1990). Recently, however, Bókkon (2008) put forward a new notion about the origin of phosphenes by suggesting that phosphene flashes result from induced or spontaneous increased overproduction of free radicals and excited biomolecules. Such overproduction is hypothesized to produce a transient excess of UPEs in cells of various parts of the visual system. When excess UPEs goes above a distinct threshold, they may emerge as phosphene flash in our mind. In other words, the brain interprets these retinal bioluminescent photons as if they originate from the external world. Bókkon's prediction about one kind of retinal phosphenes was experimentally supported by Narici et al. (2009). According to this study, free radicals induced by ionizing radiation (*cosmic particles*) can produce chemiluminescent photons via lipid peroxidation. Chemiluminescent photons are then absorbed by the photoreceptors and start the photo-transduction cascade, which results in the perception of phosphenes.

In complete darkness, a rod cell can convert a single photon into a neural signal, though cones require the coincident absorption of some photons to generate a detectable signal (Baylor et al., 1979, Vimal et al., 1989). Namely, approximately 10-30 photons are required for seeing a flash in complete darkness (Hecht et al., 1942; Field et al., 2005).

Several experiments have demonstrated that retinal phosphenes are commonly elicited by electrical, magnetic and mechanical stimuli. Here, we demonstrated that visible lights (*red, blue, and green*) can also induce ultraweak photon emission (*also called delayed*

luminescence) from isolated parts of the rat eye. Our results may suggest that retinal phosphenes result from excess bioluminescent photons, as we observed that photon emission does exist within the eye. For example, during electrical stimulation of retina, various biomolecules can undergo electron transfer reactions to form excited states and free radicals (*lipid peroxidation*) at electrodes. Then, these excited biomolecules and free radical processes can directly or indirectly produce photon emission when they return to basic states. Namely, retinal electrical induction performs a brief electrochemiluminescence process (*also known as electrogenerated chemiluminescence*) at electrodes (Chen et al., 1997).

Together, previous reports (Isojima et al., 1995; Kobayashi et al., 1999a; Kobayashi et al., 1999b; Kataoka et al., 2001; Artem'ez et al 1967; Zhang et al., 1997; Dzhafarov et al., 1987) and the current findings provide support for the hypothesis that retinal phosphenes are due to the free radical related excess UPEs in the eyes, and the brain interprets these retinal bioluminescent photons as if they are derived from our external world.

Normal vision and ambient induced delayed photons

During normal vision, the eyes are continuously exposed to powerful ambient photons that pass through various parts of the eyes. However, our experiments provide evidence for the existence of spontaneous and visible light induced delayed photon emission from various parts of *in vitro,* freshly isolated rat eyes. Therefore, ambient photons could continuously produce ultraweak delayed photon emission simultaneously from diverse parts of vertebrate eyes during normal vision. It is important to consider that under natural circumstances, ambient photons can perform much stronger delayed photon emission (*within the eyes*) than in the isolated and dark-adapted experiments described here. The significance of ambient light induced bioluminescent delayed photons within different parts of the eyes during vision is currently unknown and should be considered in future research endeavors.

In brief

In the present study, we demonstrated the existence of spontaneous and visible photon induced (*delayed luminescence*) ultraweak photon emission from *in vitro,* freshly isolated whole eye, lens, vitreous humor and retina samples from rats. These experiments may have the following implications:

i. Our results may support the bioluminescent photon concept of dark retinal noise. Specifically, the discrete dark noise of rods may be due to the ultraweak bioluminescent photons generated within the retina from natural lipid peroxidation and other free radical

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processes. It is also possible that both thermal activation processes and bioluminescent photons are simultaneously involved in the production of discrete dark noises to differing extents. It may also occur if a bioluminescent photon (*by oxidative reactions*) has lower energy (*to bleach or excite pigment*) than the activation energy of a visual pigment, the thermal energy of the molecule may be large enough to supply the deficit

ii. Here we demonstrated visible lights induced ultraweak photon emission (*also called delayed luminescence*) from isolated parts of rat eyes. Nevertheless, our results may also suggest that electrical, magnetic or other types of stimuli can induce retinal phosphenes that also result from an excess of bioluminescent photons. Thus, our experiments may indicate that induced photon emission can exist within the eyes.

iii. Retinal bioluminescent photons (retinal phosphenes) can be interpreted by the brain as if they are derived from our external world.

iv. The design of high-resolution retinal prostheses for the restoration of sight presents several unique engineering and biological challenges. Our interpretations regarding retinal discrete noise and phosphenes may lead to new notions and designs related to retinal prostheses.

v. During normal photopic (*or scotopic*) vision, vertebrate eyes are continuously exposed to powerful ambient photons that pass through various parts of the eyes. According to our results, during vision, ambient light radiation can induce permanent bioluminescent photon emission within various parts of the eyes. However, we do not know the significance or role of ambient light induced permanent bioluminescent photons during visual mechanisms.

Finally, in the future, several further studies should be conducted not only to replicate the current findings but also to extend the novel concepts of ultraweak biophotonic research. We should also consider that the ultraweak biophotonic and bioelectronic activities may be not independent biological events during neural signal processes.

4. Experimental procedures

4.1. The preparation and pre-processing of samples

Sprague–Dawley (SD) rats weighing 200 ± 15 g were purchased from the Experimental Animals Center of Tongji Medical College of Huazhong University of Science and Technology. 12 rats were employed and 7 samples (N=7) from each tissue (retina, lens, vitreous humor and whole eye) were used for experiments. All animal experiments were approved by the Animal Care Committee of South-Central University for Nationalities. Each

rat was decapitated, and both eyes were isolated quickly from fossa orbitalis. All remaining surrounding connective tissue was carefully cut away with microscissors. Under the operating microscope, the cornea from one eye was cut away with microscissors along the limbus and the lens. The vitreous body and optic cup (retina) were separated carefully. The other eye was kept intact.

4.2. Biophoton detection with a biophoton imaging system

The biophoton imaging system has been previously described (Sun et al., 2010). The system consists of a stereomicroscope (AZ100, Nikon, Japan) and an electron multiplier CCD (EM-CCD) camera (C9100-13; Hamamatsu Photonics K. K., Hamamatsu, Japan), which were set in a complete dark box (90.5 cm in length, 75.5 cm in width, 110.5 cm in height) in a dark room. The EM-CCD camera was mounted on top of the stereomicroscope and controlled by a computer and image analysis software (SimplePCI 6.0), as shown in Figure 4.
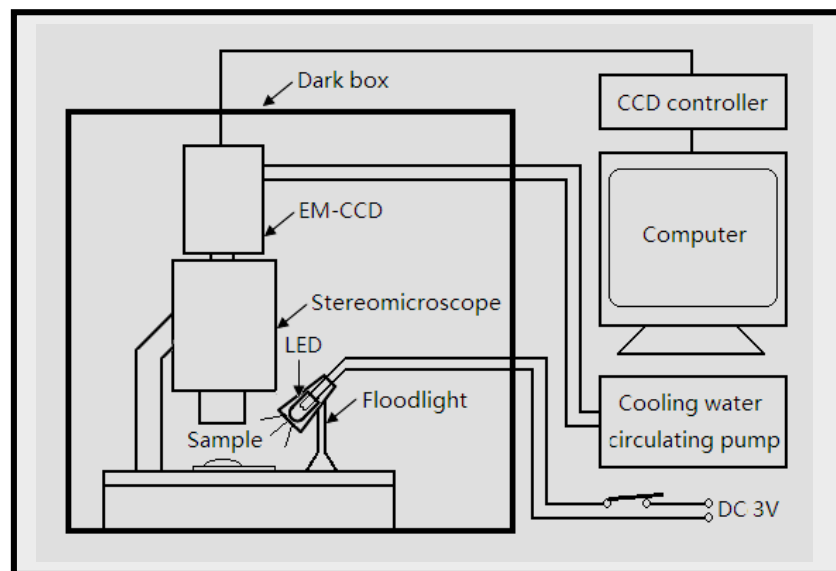

Fig. 4. Schematic drawing of the biophoton imaging system. A stereomicroscope, EM-CCD camera and LED light are set in a complete dark box. A CCD controller, computer and cooling water circulating pump are set outside of dark box (also see text).

The lens, vitreous body, optic cup (retina) and complete eye were placed on a glass slide, which was put on the specimen stage of the stereomicroscope with an objective lens (AZ-Plan Apo 1x) (see Fig. 5A). Light illumination was applied to the preparations by an LED lamp which can be replaced in different color LED lamp (red, 621 - 623.5 nm, 4,500 - 5,000 mcd; green, 515 - 520 nm, 12,000 - 14,000 mcd; blue, 460 - 465 nm, 4,000 - 5,000 mcd) supplied by a 3 V direct current (DC). The distance from the LED lamp to the samples was approximately 6 cm. A ground glass was set at the front of the LED lamp to obtain uniform illumination.

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Biophotons were detected and imaged using the EM-CCD camera in water-cool mode (in this situation, the working temperature at the CCD can be maintained as low as -90 °C). The other setup parameters for EM-CCD camera during imaging are 1200 x gain and 2 x 2 binning. The specific steps for biophoton detection were as follows: (**1**) the samples were kept in complete darkness for at least 20 min before biophoton imaging to exclude the effects of ambient light; (**2**) real-time images were taken automatically every 6 sec; (**3**) after 50 images were acquired (i.e. 300 sec basal recording), imaging was paused and the was applied to the samples by a LED lamp (first red light) for 10 sec before an additional 150 images were taken (lasting 20 min); (**4**) steps 2-3 were repeated, with the illumination time extended to 20 and 40 seconds, respectively; (**5**) steps 2-3 were repeated, with green or blue LED in place of red light illumination.

4.3. Image processing and data analysis

The images and data were analyzed by using image analysis software (SimplePCI 6.0). The specific processes as follows: (**1**) the location and size of samples were marked on a normal image with circles (Fig.5A) and this image was superposed to each image obtained for image analysis, such a treatment doesn't influence the outcome of data of image analysis; (**2**) The average intensity values (Mean gray of image) of the marked area were extracted and calculated; (**3**) the effect of cosmic rays can be eliminated by setting a gray threshold value to 10000 during image analysis.
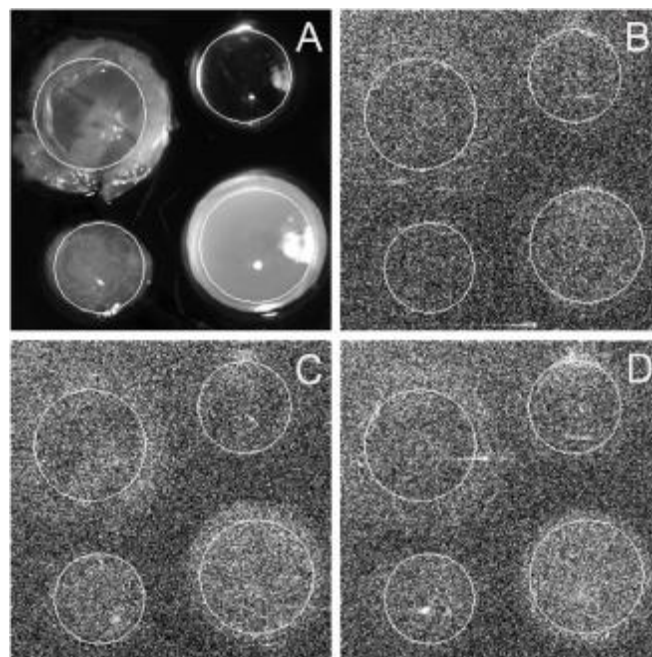

Fig. 5. Light-induced biophoton imaging and image analysis. (**A**) An image taken under the ambient light condition, showing the position of retina (upper left), lens (upper right), vitreous humor (lower left) and whole eye (lower right). (**B-C**). Representative images showing the intensity of biophotonic signals detected and imaged for 10 min by EM-CCD camera after red (B), green (C) and blue (D) light illumination (40 s) on the

retina, lens, vitreous humor and whole eye. The areas marked with circles in each sample were processed for image analysis.

4.4. Statistical analysis

All 12 rats were employed and 7 samples (N=7) from each tissue (retina, lens, vitreous humor and whole eye) were used for data analysis. Two-tailed Student's t-tests (calculated using Microsoft Excel) or one-way analysis of variance (ANOVA) were used. All summary bar graphs are presented as mean ± standard deviation, with the significance denoted as follows: *P < 0.05, **P < 0.01.

[*]**Footnote**

Delayed luminescence (DL) is the long-term ultraweak re-emission of optical photons from diverse cells, organisms, and other material if they were illuminated with monochromatic or white light (Ho et al., 2002; Popp and Yan, 2002; Kim et al., 2005). DL intensity is radically lower than the well-known fluorescence or phosphorescence. The decay time of DL is dependent on the physiological conditions of the samples and the kinds of tissues they were extracted from, as well as the intensity, duration, and spectral distribution of illumination (Kim et al., 2005).

*Abbreviations***:** UPE, ultraweak photon emissions; PMT, photomultiplier tube; EM-CCD, electron multiplier CCD camera; ROS, reactive oxygen species; RNS, reactive nitrogen species; EEG, electroencephalogram; cGMP, cyclic guanosine monophosphate; DC, direct current; V1, primary visual cortex; DL, delayed luminescence.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content.

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