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Original article

Using retinographics BPI-50 in selected eye disorders

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Summary. *Study Objectives:* The objective of this study was to assess pupillary light reflexes under pen light, red light, and blue light stimuli of different intensities in the healthy eyes of dogs and the eyes of dogs with cataracts. The study examines patients with cataracts and evaluates whether there is retinal or optic nerve damage based on the pupillary response. *Methods:* This study used 10 healthy dogs and 10 dogs with cataracts at different stages that were sedated with tomidine (5 μg/kg). The RetinoGraphics BPI-50 (Precision Illuminator) device was used for light stimulation. Light intensities comprised pen light 570 nm (nominal), red 660 nm and blue 465 nm wavelengths. *Results:* The measurements by the illuminator BPI-50 with red light and blue light source revealed that the pupil diameters of dogs with incipient and immature cataracts showed similarity with the eyes of healthy dogs. Under pen light and red light illumination, the pupil diameters of the dogs with mature and hypermature cataracts were similar to those obtained without using any light source. Under the blue light source, in turn, there was a significant reduction in pupil diameter. *Conclusion:* As an alternative to the advanced imaging techniques used in eye examination, RetinoGraphics BPI-50 (Precision Illuminator) can be used to assess pupillary light reflex for pre-diagnostic purposes. A blue light source can be used in mature and immature animals in cases where the pupillary reflex created by pen light is insufficient.

Key words: BPI-50, Cataract, Melanopsin, Pupillary light reflex, Retina

Introduction

The autonomic nervous system allows pupil constriction through parasympathetic stimulation (miosis) in bright light and when looking at something close, and pupil dilation (mydriasis) through sympathetic stimulation in the dark and when looking at a faraway object. The afferent pathway of the light reflex is the optic nerve, and the efferent pathway is the parasympathetic fibers in the *Nervus oculomotorius* (*N. oculomotorius*) (1). The pupillary light reflex occurs with sympathetic and parasympathetic stimulation. The reflex extends from the retina to the pretectal nucleus in the midbrain with parasympathetic stimulation. Upon the stimulation of photoreceptors by light, the reflex starts in the retina. Impulses from the nasal half of the retina cross in the chiasma opticum and reach the pretectal nucleus on the opposite side through tractus opticus. Impulses from the temporal half of the retina, in turn, reach the pretectal nucleus on the same side without crossing in the chiasma opticum. It is the intern neuron that connects the pretectal nucleus to both Edinger-Westphal nuclei. When light is directed in one pupil, both pupils constrict symmetrically and simultaneously. Fibers connect the Edinger-Westphal nucleus to the ciliary ganglion. These fibers are parasympathetic fibers in the inferior branch of *N. oculomotorius* and form the efferent limb. After entering the *N. oculomotorius orbita*, the efferent fibers of the pupillary light reflex (PLR) leave the nerve and reach the ciliary ganglion via the inferior branch to the inferior oblique muscle. It is located within the short posterior ciliary nerves by establishing a connection between the ciliary ganglion and the sphincter pupillae. This induces miosis (2,3). With sympathetic stimulation, the fibers originating from the posterior hypothalamus descend without crossing, making

multiple synapses in the mesencephalon and pons, and terminate in the ciliospinal center of the spinal cord. It extends from the ciliospinal center to the superior cervical ganglion by joining the cervical sympathetic chain at the level of the inferior cervical ganglion. It runs along the internal carotid artery, enters the cranium and joins the ophthalmic branch of the trigeminal nerve in the sinus cavernosus. Sympathetic fibers reach the ciliary body, pupillary dilator, iris melanocytes, and Müller's muscle via the nasociliary nerve and long ciliary nerves, inducing mydriasis (2).

In recent years, research has focused on understanding the physiological basis of PLR activity (4,5). The discovery of a vitamin A-based photosensitive pigment (melanopsin) in retinal ganglion cells extending to the midbrain structures that mediate the PLR activity, and the presence of intact PLR activity in eyes with an almost completely damaged photoreceptor layer provide a basic explanation for circadian rhythm regulation and visual function. Normally, the PLR activity is clinically tested using non-chromatic white light stimuli of different light intensities (5-7). Although melanopsin-containing retinal ganglion cells (ipRGC) can be activated by rod and cone photoreceptors in the outer retina (8,9), they contain opsin melanopsin (Opn4) as in invertebrates, which makes them directly sensitive to light (8,10,11). Melanopsin cells reflect the pupillary light reflex (10,12,13) to the olivary pretectal nucleus (10,12,14,15). M1 ipRGCs, which reflect the transcription factor Brn3b to the olivary pretectal nucleus in rats, are believed to be necessary for the pupillary light reflex (13). Different types of melanopsin cells have also been identified in macaques and humans (16,17), but the research is ongoing about their roles in different non-visual light responses. Melanopsin is required for pupillary light responses in the lesser-mole rat (5,6), but visual photoreceptors may mediate the pupillary light reflex in rats' melanopsin (18).

Pupillary light reflex and other non-visual light responses disappear only when the rod, cone, and melanopsin signaling pathways are impaired simultaneously (5,6). In addition, the selective ablation of melanopsin containing ipRGCs severely reduces pupillary responses to light (most, if not all). The light information is directed from the outer retinal photoreceptors to the olivary pretectal nucleus through several thousand melanopsin cells widely distributed throughout the retina (10). Differences in anatomical location and response characteristics between rod/cone photoreceptors, and melanopsin have led to increased interest in using the pupillary light reflex to detect the loss of photoreceptor function in retinal and optic nerve diseases. If the function of IpRGCs and conventional RGCs (retinal ganglion cells) is impaired due to a particular disease, the pupillary light responses can be used to predict damage to the afferent pathway, including image formation/vision. Given that rods, cones, and melanopsin play different roles in mediating the pupillary light reflex (20,21), the light stimuli can preferably act in a manner that ensures specific reading of their functions by stimulating preferably one or more photoreceptor types. This has led to an increased tendency towards chromatic pupillometry (also called color pupillometry or selective wavelength pupillometry) methods, which allow measuring pupillary responses at different wavelengths and light intensities to discriminate rod, cone, and melanopsin involvement in the pupillary light reflex. The pupillary light reflex is mediated by light-sensitive ipRGCs that extend from rods and cones. Melanopsin-dependent pupillary light responses are sensitive to short wavelengths, have a higher activation threshold, and are much slower than rod- / cone-mediated responses (22). In addition, there are reports of pRGCs being maximally sensitive to short-wavelength light, the transmission of which is reduced by cataracts (23).

When the pen light source is used in mature and hypermature cataract cases, the pupillary light reflex can be detected in some cases but not in others. Therefore, in dogs diagnosed with cataracts, preoperative electroretinography (ERG) is used to determine whether there are lesions and vision loss in the retina. Due to the high cost and lack of portability of ERG, we believe that BPI-50 (RetinoGraphics) can be used for retinal examination as it has the same function. The aim of the present study was to assess the pupillary reflexes of dogs with different stages of cataract under pen light as well as red and blue light stimuli of different intensities created with BPI-50.

Materials and Methods

All animal studies were carried out at Pamuk Veterinary Therapy Center. In this study, 10 healthy

dogs and 10 dogs with different stages of cataract were used. Before study inclusion, these animals had an ocular examination (intraocular pressure measurement, slit-lamp biomicroscopy, and indirect ophthalmoscopy) to exclude possible ocular diseases. All the dogs were mildly sedated with an intramuscular dose of 5 μg/kg tomidine (Provet, TOPKIM, ALIVIRA). This dose is lower than the sedation dose prescribed for dogs because a higher sedation dose causes miosis and poor response to light stimuli. The animals were kept in cages under mesopic conditions (dim light) in a quiet room for a minimum of 15 minutes after sedation. For light stimulation, pen light with an intensity of 570 nm (nominal), red 660 nm and blue 465 nm wavelengths were applied. The application time for both lights was 5 s at low intensity and 10 s at high intensity. The study examined patients with cataracts using RetinoGraphics BPI-50 (Precision Illuminator) and evaluated whether there was retinal or optic nerve damage based on the pupillary response. BPI-50 is a device that provides a precision red and blue light source, allows examination without any invasive procedure, costs less than ERG, (6) is easy to use, does not require adjustments, and is repeatable. It is microprocessor controlled. It has a photometrically balanced illuminance (CIE 1931 photopic luminosity function). It can be easily distinguished by the eye and the light can be aligned exactly where it is desired.

Statistical Analysis

The data obtained in the study were analyzed with the SPSS for Windws program. Independent samples *t*-test was used for comparing normal, pen light, blue light, and red light between groups with cataracts and healthy groups. Statistical significance level was 0.05. This study was approved by decision number 180 and dated 29/11/2017.

Results

Mean Intraocular Pressure (IOP) and PLR Values

In Healthy Dogs: According to the measurement by Tono-Pen XL, the mean intraocular pressure of 10 healthy dogs under tomidine sedation was 16.81 mm/g, and the pupil diameter without any light exposure was 9.02 mm. The mean pupil diameter in PLR was 3.93 mm under pen light, 4.69 mm under blue light and 5.49 mm under red light. The IOP and PLR values of the healthy dogs are provided in Table 1.

In Dogs with Cataracts: Among 10 dogs, the examination under tomidine sedation revealed incipient cataract in two, immature cataract in two, mature cataract in four, and hypermature cataract in two dogs. The mean IOP was 17.35 mm/Hg, 17.15 mm/Hg, 16.68

Table 1. IOP and PLR values in healthy dogs

No	Stages of cataract	IOP (mm/Hg)	PLR (normal) Pupil diameter (mm)	PLR (pen light) Pupil diameter (mm)	PLR (blue light) Pupil diameter (mm)	PLR (red light) Pupil diameter (mm)
1	Incipient	17.8	8.4	4.6	4.9	6.1
2	Incipient	16.9	8.5	4.4	5.1	6.2
3	Immature	15.2	8.9	4.9	5.3	6.6
4	Immature	19.1	8.8	4.8	5.1	6.4
5	Mature	16.3	9.6	9.3	4.3	9.1
6	Mature	16.7	9.7	9.3	4.2	9.6
7	Mature	18.4	9.6	9.1	9.2	9.4
8	Mature	18.3	9.8	9.2	5.1	9.0
9	Hypermature	15.9	10.0	9.7	4.3	9.7
10	Hypermature	19.1	9.9	9.5	4.7	9.5

Table 2. IOP and PLR values in dogs with different stages of cataract

No	IOP (mm/Hg)	PLR (normal) Pupil diameter (mm)	PLR (pen light) Pupil diameter (mm)	PLR (blue light) Pupil diameter (mm)	PLR (red light) Pupil diameter (mm)
1	19.6	8.1	4.3	5.1	6.2
2	18.7	7.9	3.9	4.3	5.9
3	15.9	8.2	4.1	5.2	6.0
4	16.2	8.4	3.8	4.3	4.9
5	17.8	7.8	4.0	4.7	5.1
6	17.4	8.0	4.3	4.9	6.3
7	14.4	8.2	3.4	4.7	5.4
8	15.8	8.1	3.6	4.4	5.0
9	17.2	7.6	4.0	4.6	5.2
10	18.1	7.9	3.9	4.7	4.9

mm/Hg, and 17.50 mm/Hg for incipient, immature, mature, and hypermature stages, respectively. The mean pupil diameter was 8.45 mm, 8.85 mm, 9.68, and 9.95 mm in incipient, immature, mature, and hypermature cataract cases, respectively without any light exposure; 4.45 mm, 4.85 mm, 9.20 mm, 9.60 mm, and 5.00 mm under pen light; 5.00mm, 5.20 mm, 6.88 mm, and 4.50 under blue light, and 6.15 mm, 6.50 mm, 9.28, and 9.60 mm under red light. The IOP and PLR values of the dogs with cataracts are provided in Table 2.

Findings regarding the comparison of cataract and healthy dogs in terms of normal, pen light, blue light and red light are shown in Table 3. According to the test results, there was no significant difference between the groups in terms of IOP and PLR blue light values, while significant differences were found between the groups in terms of PLR normal, pen light and red light.

Discussion and Conclusion

The pupillary light reflex is a significant indicator of retinal and optic nerve function after light stimulation. PLR is of significance in clinical practice because it assesses afferent defects [retina, optic nerve, pregeniculate visual pathways (chiasma, optical path, and midbrain)] due to any disease, and provides information about retina and visual pathway functions (24). Pupillometry allows for precise measurement of changes by observing pupillary responses. Chromatic pupillometry is used in dogs to assess the visual pathway and to measure specific color light responses (24-27). Melanopsin, the photopigment of IpRGC, is an important structure in the use of blue light PLR to distinguish diseases affecting the inner retina, optic nerve, and central nervous system, with a spectral sensitivity of approximately 480 nm (10,28). Chromatic pupillometry utilizes red and blue light stimuli that allow for detailed testing of retinal cell subpopulations. Pupillary constriction, assessed by dim and bright blue light stimuli in the dark (scotopic dim blue [scDB] and scotopic bright blue [scBB]), measures rod- and ipRGC-mediated functions, while bright red light stimulus with a blue background (photopic bright red [phBR]) specifically measures cone function (29,30).

Our findings reveal a mean pupil diameter of 9.02 mm in healthy dogs under sedation, without any light exposure. The mean pupil diameter was 3.9 under pen light (570 nm), 4.69 under blue light, and was 5.49 under red light (660 nm). These values show that different wavelengths of light affect the PLR pathways in the eye, resulting in different pupil diameters. Similarly, rods, cones, and ipRGCs have been reported to induce pupillary reflexes, especially in response to blue and red light stimuli in dogs (24).

In the present study, the pupil diameter of animals with incipient and immature cataracts who were not exposed to any light source was close to that of healthy animals. The pupil diameter was greater in the animals

Table 3. Comparison of cataracts and healthy dogs in terms of IOP and PLR

Parameters	Groups	n	Mean	SD	p
IOP (mm/hg)	Healthy	10	17.11	1.55	0.695
	With cataracts	10	17.37	1.36	
PLR (normal) Pupil diameter (mm)	Healthy	10	8.02	0.22	0.001*
	With cataracts	10	9.32	0.60	
PLR (pen light) Pupil diameter (mm)	Healthy	10	3.93	0.28	0.001*
	With cataracts	10	7.48	2.42	
PLR (blue light) Pupil diameter (mm)	Healthy	10	4.69	0.31	0.274
	With cataracts	10	5.22	1.45	
PLR (red light) Pupil diameter (mm)	Healthy	10	5.49	0.55	0.001*
	With cataracts	10	8.16	1.59	

*p<0,05

with mature (9.68 mm) and hypermature (9.95 mm) cataracts than that of the healthy animals (9.02 mm).

In this study, IOP and PLR values of cataracts and healthy dogs were compared. While there was no statistical difference between IOP and blue light values, PLR was normal, pen light and red light values were higher in dogs with cataracts. In addition, the pupil diameters of dogs with incipient and immature cataracts, and healthy dogs exposed to pen light, blue light, and red light were similar. However, the pupil diameter under pen light was 9.20 mm and 9.60 mm in dogs with mature and hypermature cataracts, respectively. These values were greater than those of the healthy animals. While there was no significant difference between the groups in terms of IOP and PLR blue light values, significant differences were found between the groups in terms of PLR normal, pen light and red light. It was observed that the average values of dogs with cataracts were higher in these parameters.

The reason for this is that the lens of a healthy animal is transparent and the eye can receive light, but the mid-wavelength pen light cannot reach the posterior segment of the eye due to the loss of transparency in mature and hypermature cataracts, and photoreceptors in the posterior segment that stimulate the pupillary reflex cannot use this wavelength. Therefore, the pupil was larger in dogs with mature and hypermature cataracts. On the other hand, the mean pupil diameter under blue light differed between eyes with mature cataract (6.88 mm) and the eyes of healthy animals (4.69); however, this difference was due to the large pupillary diameter obtained from case 7. The large pupil diameter of case 7 suggests the presence of a lesion in the melanopsin-containing photoreceptor. In this sense, the values of eyes with mature cataracts under blue light are in parallel with those of healthy animals under the same light. The diameter under blue light was similar between the eyes with hypermature cataracts (4.50 mm) and the eyes of healthy animals (4.69 mm).

Grozdanic et al. (24) have demonstrated that the (rod- / cone-mediated) PLR components of melanopsin and non-melanopsin could be separated by using light stimuli (blue versus red light response) with different wavelengths. Since melanopsin sensitivity is close to 480 nm wavelength (blue light), blue light stimuli with adequate light intensity can be used properly to assess the function of retinal ganglion cells containing melanopsin that are not in the rod/cone extension (4), and thus the optic nerve function. The use of red light (630 nm) that has a wavelength not suitable for melanopsin sensitivity can be properly used to test the cone photoreceptor-mediated component of PLR (4,11). Over the past few years, the characteristics of PLR have been extensively examined in various dogs with retinal and optic nerve diseases, revealing that the chromatic assessment of PLR is an important test for two reasons. The first is to distinguish between retinal and optic nerve diseases. The second is that PLR together with electroretinography can

accurately localize the pathological process. In dogs with different forms of retinal detachment, usually weak or absent PLR with the red stimulus is characterized only by near-normal PLR with blue stimulus, due to primary RPE-photoreceptor abnormalities and intact inner retinal function (31). In our study, the pupil diameters of eyes with mature and hypermature cataracts were similar under red light and pen light exposure. An adequate PLR was not developed under red light and pen light exposure. However, the blue light exposure produced a PLR similar to that of healthy dogs. Considering these values established in our study, it is necessary to investigate cataract cases in terms of retinal detachment.

The present study can be used to develop effective diagnostic strategies for similar diseases in various animals based on the chromatic assessment of PLR activity in different breeds of dogs with different stages of cataracts.

Inability to examine the posterior segment of the eye directly and indirectly, especially in patients with mature and hypermature cataracts, is an important problem since the lens loses its transparency. Advanced imaging techniques such as ERG or computed tomography (CT) and magnetic resonance (MR) are required to understand whether there are visual functions. Portable electroretinography devices such as BPI-50 have been used in the measurement of PLR activities during the last few years. The findings of our study indicate that with this device a preliminary diagnosis can be established regarding the visual function of dogs with mature and hypermature cataracts. The study also suggests that the device can provide partially enlightening information in posterior segment diseases of the eye. However, the lack of adjustment to the wavelength range is a disadvantage of the device.

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