Quantitative assessment of pupillary light reflex in normal and anesthetized dogs: a preliminary study

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ABSTRACT.   The purpose of this study was to quantitatively assess the pupillary light reflex (PLR) in normal and anesthetized dogs using a pupillometer. Eleven dogs (20 eyes) of various breeds were included. PLRs were measured with a handheld pupillometer in dim light before and during anesthesia. Anesthesia was conducted with atropine, xylazine and ketamine. Parameters of pupillometry included neurological pupil index (NPi), pupil size, percent of change (%CH), latency (LAT), constriction velocity (CV), maximum constriction velocity (MCV) and dilation velocity (DV). NPi,%CH, CV and MCV were significantly decreased during anesthesia compared with the pre-anesthesia data. The results suggest that atropine-xylazine-ketamine combination anesthesia depresses the PLR. Additionally, this study demonstrates the feasibility of the use of a pupillometer in dogs.
KEY WORDS: canine, pupillary light reflex, pupillometer

The pupillary light reflex (PLR) is the constriction of the pupil in response to light striking the retina. Stimulation of the retina is followed by transmission of information along the optic nerve and through the optic chiasm and optic tract to the pretectal area of the brain and the parasympathetic nucleus of oculomotor nerve. The efferent parasympathetic axons leave the oculomotor nuclei with the motor axons of the oculomotor nerve. The reflex arc ultimately results in stimulation of the smooth muscles of the iris sphincter muscle to cause pupil contraction [7]. Because this reflex is an integrated response of both the eye and the brain, assessment of the PLR has long been used to assess iris innervation and function as well as for ophthalmic and neurologic disease diagnosis in both human and veterinary medicine [8, 12]. However, routine clinical examinations usually include only gross observations of the PLR, which are largely qualitative. Such qualitative PLR assessments can be used only to localize lesion when substantial deficits in retinal or autonomic function are present, and they cannot be used to quantify the amount of function lost or present [11].

Detailed quantitative assessments of the PLR have been performed in human medicine. The sizes, speed of reactivity and abnormalities in pupil shape have been studied for almost 100 years [1]. As a result, pupillometry has been widely used in the evaluation of numerous pathological findings in neurology and critical care as well as in ophthalmology [1, 2]. Recently, a well-designed clinical study introduced a pupillometer that greatly facilitates quantitative pupillometry in humans [1]. The NeurOptics pupillometer, one of the automatic pupillometers, uses a handheld infrared system and tracks and analyzes pupil dynamics quickly. This pupillometer accurately, reliably and quantifiably measures pupillary reactivity [2]. Unfortunately, few studies with this pupillometer have been reported in veterinary medicine [3, 10, 11].

Therefore, the purposes of this study were 1) to evaluate the feasibility and utility of this pupillometer in dogs and 2) to determine if the pupillometer can possibly detect different PLR responses between conscious and unconscious dogs anesthetized with an atropine-xylazine-ketamine anesthetic protocol.

Eleven healthy small breed dogs presented for routine neutering (n=6) and tooth scaling (n=5) under general anesthesia at Ji Dong Beom Animal Medical Center were included in this study. All animal studies were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Only animals with normal results in physical examination, preanesthetic blood profiles and radiographs, as well as complete neurologic and ophthalmic examinations, including the Schirmer tear test (STT), rebound tonometry and slit lamp biomicroscopy (Slit Lamp BQ 900®, Haag-Streit, Bern, Switzerland) to observe the anterior segment and fundic and neuro-ophthalmic evaluation (menace response, dazzle reflex, palpebral reflex and PLR), were included in this study.

PLRs of the dogs were measured with a handheld pupillometer (NPI-100, NeurOptics, Inc., Irvine, CA, U.S.A.) with an internal time of 5 to 10 min on each eye. The pupillometer was placed in front of the eye, and pupil light measurement began with a flash of light of fixed intensity and duration effectively stimulating the pupil light constriction

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reflex. The measurement lasts 3.2 sec, allowing for full or partial recovery of the pupil size after light constriction. At the end of the measurement, a screen was presented showing the results.

Measurement of the PLR was performed twice in dim light, before and during anesthesia. Following the initial data acquisition, all animals were premedicated with atropine sulfate (0.02 mg/kg, Atropine sulfate, Jeil Pharmaceutical, Seoul, Republic of Korea) subcutaneously 15 min prior to induction of anesthesia with xylazine (1 mg/kg, Rompun®, Bayer, Seoul, Republic of Korea) and ketamine (4 mg/kg, Ketamin 50®, Yuhan, Seoul, Republic of Korea) intravenously. PLR was measured under the same conditions before and after anesthesia. The postinduction PLR was measured after the animal had reached a steady plane of general anesthesia with no response to toe pinch 5 min after ketamine and xylazine injection. The parameters recorded during pupillometry included neurological pupil index (NPi), pupil size (MAX and MIN), percent of change (%CH), latency (LAT), constriction velocity (CV), maximum constriction velocity (MCV) and dilation velocity (DV) (Fig. 1). The pupil size variables measured by the pupillometer and their corresponding descriptions are listed in Table 1.

The data from before and during anesthesia were compared using the paired t-test by SPSS (11.0 Chicago, IL, U.S.A.), and $P<0.05$ was considered statistically significant.

The breeds represented included the Maltese (n=7), shih Tzu (n=2), schnauzer (n=1) and toy poodle (n=1). The average age was 4.91 years old (1–9 years old).

The results for the anesthetic parameters before and during anesthesia are presented in Table 2. The NPi was significantly decreased in anesthetized dogs compared with the baseline, from 4.10 ± 0.52 to 2.03 ± 0.69. In addition, the pupil constriction percentage (%CH) also decreased significantly during anesthesia, from 26.6 ± 6.57 to $13.4 \pm 5.74\%$. The maximum pupil diameter was decreased (from 9.60 ± 0.57 to 8.22 ± 1.08 mm), and the minimum pupil diameter increased (from 7.06 ± 0.91 to 7.14 ± 1.24 mm); however, there was no significant difference between before and during anesthesia (P value >0.05).

Changes in velocity were detected as well. The maximum and average constriction velocities decreased significantly during anesthesia; the maximum constriction velocity de-
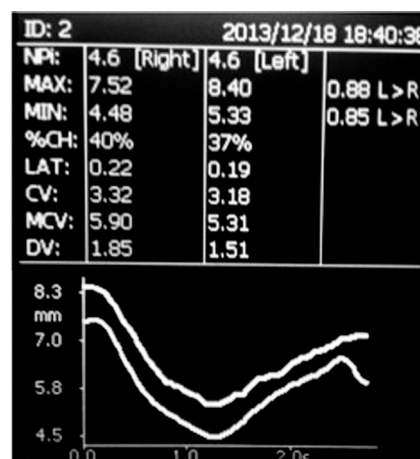

Fig. 1.   Parameters measured with the pupillometer in the dogs.

creased from 5.83 ± 1.40 to 3.65 ± 1.04 mm/sec, and the average constriction velocity decreased from 3.59 ± 0.85 to 2.19 ± 0.90 mm/sec.

There were no significant decreases during anesthesia compared with the baseline in latency (0.28 ± 0.07 vs. 0.25 ± 0.07 sec) and dilation velocity (1.63 ± 0.55 vs. 1.03 ± 0.71 mm/sec).

Few PLR studies have been reported in veterinary medicine. The first study was performed in beagle dogs in the 1970s [10]. That study was conducted with a fundus camera, and the pupil diameter was measured from a photograph using a calibrated graticule. Whiting *et al.* reported quantitative assessment of PLR in miniature long-haired dachshunds using a custom apparatus and recording system [11]. Their system has the advantage of using variable light intensities; however, the relatively long data acquisition time required chemical restraint of the subjects. Another study was performed using blue and red light to evaluate the pupil diameter of healthy and diseased canine eyes [3]. That study revealed that the pupil in Sudden Acquire Retinal Degeneration Syndrome (SARDs) patients does not constrict in red light but does constrict in blue light. Separate red and blue lights and a recording system were used in that study.

Table 1.   Pupil size variables measured by the pupillometer and their corresponding descriptions

Parameters	Unit of Measure	Definition/Calculation
NPi™ (neurological pupil index™)	Scalar value 0–5	Algorithm that takes all variables below as inputs and compares them with a normative model to give a composite score of pupillary response
MAX/MIN	mm	MAX=initial resting pupil size MIN=pupil size at peak of the constriction
%CH	%	Constriction% or percentage change ([MAX−MIN] / MAX)
LAT	Seconds	Latency=time difference between initiation of retinal light stimulation and onset of pupillary constriction
CV/MCV	mm/sec	Average constriction velocity (CV)=amount of the constriction divided by duration of constriction Maximum constriction velocity (MCV)=peak value of velocity during constriction
DV	mm/sec	Average dilation velocity (DV)=amount of pupil size recovery (after the constriction) divided by duration of recovery

Table 2. Result from pre and intra anesthetized dogs

Parameter Measured	Baseline	Anesthesia	P value
Npi™	4.10 ± 0.52	2.03 ± 0.69	0.032*
MAX (mm)	9.60 ± 0.57	8.22 ± 1.08	0.061
MIN (mm)	7.06 ± 0.91	7.14 ± 1.24	0.765
%CH (%)	26.6 ± 6.57	13.4 ± 5.74	0.001*
LAT (sec)	0.28 ± 0.07	0.25 ± 0.07	0.133
CV (mm/sec)	3.59 ± 0.85	2.19 ± 0.90	0.041*
MCV (mm/sec)	5.83 ± 1.40	3.65 ± 1.04	0.035*
DV (mm/sec)	1.63 ± 0.55	1.03 ± 0.71	0.09

* P value <0.05.

A NeurOptics pupillometer, a handheld infrared system that automatically tracks and analyzes pupil dynamics over a 3-sec period, was used in this study to evaluate pupil size and reactivity. Pupil light measurement with a flash of light of fixed intensity (180 microwatts for a total duration of 667 msec) and duration effectively stimulates the pupil light constriction reflex. The measurement takes 3.2 sec, allowing for full or partial recovery of the pupil size after constriction. Tracking of the pupil is performed at over 30 frames per sec. This short measurement period is a big advantage in veterinary patients, because most of the animals do not require chemical restraint. In fact, we appreciated no differences in the ability to conduct measurements with this pupillometer between conscious and anesthetized animals in this study.

This pupillometer was specifically designed to minimize possible interobserver variability in pupillary evaluation [2]. As compared with other pupillometers, the NeurOptics pupillometer shows the best interobserver agreement and higher inter- and intra-observer repeatability [2, 9].

The NPi algorithm was developed to quantify pupillary reactivity and remove subjectivity from the assessment. Pupil variables including size, latency, constriction velocity and dilation velocity are parameters of the NPi algorithm. Table 1 lists the pupil variables measured and how they are calculated for NPi. Each variable from an individual pupil measurement taken by the pupillometer is compared against the mean of a reference distribution of healthy human subjects for the same variable, and the difference is taken and standardized with by the corresponding standard deviation. Finally, the set of all the standardized differences (or z-scores) is combined such that is falls into a scale set between 0 and 5. An NPi value closer to 5 is considered more "brisk" than an NPi value closer to 3. An NPi score <3 describes an abnormal pupillary light reflex (i.e., weaker than a normal pupil response, as defined by the multidimensional normative model, or "sluggish"), with a value of 1 being more abnormal than a value of 3. This parameter was used in this study to find the relationship between NPi and intracranial pressure. The NPi scales in patients with high intracranial pressure tend to be decreased [2]. Although the NPi values are significantly different between awake and anesthetized dogs, the NPi scales are based on human subjects as described above. Therefore, this parameter should be used as a guide until further studies establish scales for veterinary patients.

As presented in the results, anesthesia resulted in significant differences in several parameters of the pupillometry. Some parameters, including NPi,%CH and the velocity of the constriction, decreased significantly in anesthetized dogs. All of the medications used for anesthesia in this study, atropine, ketamine and xylazine, have a reported mydriatic effect on the pupil in dogs. Atropine, an anticholinergic agent, is well known to induce mydriasis after the topical use in dogs. Recently, Kovalcuka and colleagues reported that systemic use of atropine also induces a significant increase in pupil diameter in beagles [6]. Moreover, ketamine and xylazine also have a mydriatic effect on canine pupils [4, 5]. Therefore, the significant decrease in the change in pupil size in anesthetized dogs may result from the inhibition of pupil constriction in terms of size and velocity by the combination of the three drugs.

This study has several limitations. First, NPi scales should be used with caution in veterinary medicine. Because this device is designed for use in humans, the NPi scales are derived from human data, and a direct comparison may not be appropriate. Although we demonstrated a significant difference in NPi between the normal and anesthetized animals, the number of subjects was small, and the experiment was only designed to test a single set of conditions in normal dogs. Additional data points may more precisely characterize differences in pupil parameters. Another limitation of this study is that the instrument used is unable to assess pupils over 10 mm in diameter. Previous studies have reported the normal pupil diameter of conscious dogs to be 8.3 ± 0.6 mm [3] or 10.06 ± 0.54 mm [11], while the pupil size was 9.60 ± 0.57 in this study. There will be a subset of normal dogs in which this instrument will not be able to accurately obtain measurements. PLR measurement is possible for most dogs; however, measurement of the mydriatic pupil may be limited in some cases. In addition, this study was conducted with only a small number of normal subjects; further studies are needed to assess the utility of this pupillometer in many patients with retinal and neurological diseases and a maximum pupil diameter of 10 mm.

The device was able to detect differences in PLR parameters between dogs that were conscious and those anesthetized with a combination of atropine, xylazine and ketamine. Although there are limitations to use of the NPi pupillometer and interpretation of the results because it was originally designed for human subjects, its use is feasible in canines, especially if further studies are conducted.

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