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Effect of mydriasis with topical rocuronium bromide on electroretinography in domestic pigeons (*Columba livia*)

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ABSTRACT. This study aimed to investigate the effect of mydriasis using topical rocuronium bromide on electroretinography (ERG) in domestic pigeons (*Columba livia*). Scotopic mixed rod and cone, photopic cone, and photopic flicker ERG were performed on nine eyes of nine healthy adult pigeons under sedation. Each pigeon underwent two sets of ERG recordings. First, without the induction of mydriasis (control) and the second time with the induction of mydriasis using topical rocuronium bromide (treatment). The results were compared using either the Student's *t*-test or Wilcoxon rank-sum test, where a *P*-value of <0.05 was considered statistically significant. No significant differences were observed in the a- and b-wave implicit times and amplitudes during scotopic ERG between the two groups. The a- and b-wave amplitudes in the photopic cone were significantly higher in the treatment group (63.83 ± 32.33 and 191.75 ± 94.46 µV) compared to the control group (46.15 ± 27.60 and 116.76 ± 70.65 µV; *P*=0.045 and *P*=0.032, respectively). The photopic flicker amplitude was also significantly higher in the treatment group (76.23 ± 48.56 µV) than in the control group (42.18 ± 31.18 µV; *P*=0.044). No statistically significant differences were observed in the photopic cone and flicker implicit times between both groups. In conclusions, mydriasis induced by rocuronium bromide in pigeon resulting in higher amplitudes during the photopic ERG but not scotopic ERG.

KEY WORDS: avian, electroretinography, mydriasis, pigeon, rocuronium bromide

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Electroretinography (ERG) measures the electrical potential generated by the retina in response to changes in illumination [10]. It is the most widely used electrodiagnostic test in veterinary ophthalmology and is commonly used to diagnose outer retinal disease [7]. ERG is considered a useful diagnostic tool to investigate retinal function as it can be performed non-invasively [7]. However, in order to obtain a reliable ERG recording, several technical aspects must be addressed, including an equal light stimulation of photoreceptors in all parts of the retina [7, 8].

During the ERG recording, a mydriatic pupil is essential because a miotic pupil can reduce the amount of light that reaches the retina, which will reduce the amplitude and prolong the implicit time obtained during the ERG [7]. However, inducing mydriasis in birds is considered challenging because the birds' iris has a different muscular system, and the mydriatic agent commonly used in mammals does not work [14]. Because of that, several reports on electroretinography on birds had been performed without the induction of mydriasis [12, 15, 16, 25, 26]. Recently, rocuronium bromide had been reported to be a reliable and safe mydriatic agent in several species of birds [2–6, 23] including pigeon [27]. This study was conducted to investigate the difference between ERG recordings with and without pupil dilation in a small-eyed bird such as domestic pigeons (*Columba livia*).

MATERIALS AND METHODS

Experimental animals

Nine healthy adult domestic pigeons (*Columba livia*) without retinal abnormalities of undetermined sex were used in this study. The eyes and periocular region were examined for gross abnormalities in a well-lit room. Intraocular pressure (IOP) measurements were performed using a rebound tonometer (TonoVet; Icare; Tiolat, Helsinki, Finland), and examinations of the adnexa and anterior segment were performed with a slit-lamp biomicroscopy (Keeler PSL One Portable Slit Lamp; Keeler Ltd., Windsor, UK). Fundus imaging was taken using a hand-held retinal camera (Genesis; Kowa, Tokyo, Japan). This study was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-190922-1).

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ERG recording

ERG recordings were performed on a different day from the general eye examinations to avoid the excessive light exposure to the retina that might interfere with ERG recordings. Each pigeon underwent two sets of ERG recordings on the right eye only. The first set of ERG recordings was performed without mydriasis induction, which acted as the control group. After the first ERG set was performed, mydriasis was induced before the second dark adaptation stage for the second set of ERG recordings or the treatment groups. Mydriasis was induced using a single topical instillation of 0.20 mg/20 µl rocuronium bromide (Rocunium Inj. 10 mg/ml, Hana Pharm. Co., Ltd., Kyonggi-Do, Korea) on the ocular surface. Because the bone separating the right and the left eyes of the pigeon is so thin, a light shone in one eye can be seen on the contralateral eyes. Therefore, in order to avoid the possibility of the first ERG test (of the first eye) interfering with the second test (of the second eye), only the right eye was used in this study.

The ERG recordings were performed under sedation using 10 mg/kg of alfaxalone (Alfaxan 10 mg/ml, Jurox, Rutherford, Australia) injected intramuscularly into the pectoral muscle before the dark adaptation stage. Three ERG recordings: 1) scotopic (dim-light) mixed rod and cone response, 2) photopic (bright light) cone response, and 3) photopic flicker response were performed using a RETIport (Roland Consult Stasche & Finger GmbH, Brandenburg, Germany). A 0.25 mm goldring electrode (3325RC; Roland Consult Stasche & Finger GmbH) was used as the corneal electrode, and platinum subdermal needle electrodes (model F-E2, Astro-Med Inc., West Warwick, RI, USA) were used as the ground and reference electrodes. The ground electrode was placed subcutaneously on the apex of the occiput, and the reference electrode was placed at approximately 0.3–0.5 cm lateral to the lateral canthus of the eye. Proparacaine hydrochloride (Alcaine, Alcon, Vilvoorde, Belgium) eye drops were applied to the cornea as a topical anesthetic before placing the corneal electrode.

The pigeons were dark adapted for 20 min before the scotopic mixed rod and cone response was obtained at a stimulus intensity of 3.0 cd sec/m^2. Four responses from flashes with an interval of 17 sec were averaged. The pigeons were then light adapted for 10 min using a background light of 30 cd/m^2, and the photopic cone and flicker responses were recorded. The flash presented at a stimulus intensity of 3.0 cd sec/m^2 were delivered with a stimulus frequency of 2 Hz for the photopic cone and 27.78 Hz for the flicker. The pigeons were monitored for signs of systemic side effects from the topical application of rocuronium bromide throughout the ERG procedure until the next day.

Statistical analysis

The data obtained for the scotopic mixed rod and cone and photopic cone responses were the a- and b-wave implicit times and amplitudes. For the photopic flicker response, the data obtained were the peak implicit time and amplitude. Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). A Student's *t*-test was performed to compare the data (a- and b-wave implicit times and amplitudes) between the groups, except for the data of a-wave implicit time from photopic cone response, which was analyzed using the Wilcoxon rank-sum test (Mann-Whitney *U* test). A *P*-value of <0.05 was considered statistically significant.

RESULTS

The general ophthalmic examinations of the experimental pigeons were all normal. No retinal lesions were observed on the funduscopic examination. IOP was ranged from 9 to 14 mmHg. The pigeon's pupil size without the application of rocuronium bromide were 3.05 ± 0.24 mm in diameter. On the second set of ERG recordings, after the dark adaptation stage, the pupil was checked under dim red light (not directly shone to the eye) to ensure successful pharmacological pupil dilation. However, pupil measurement was only done after the ERG recording had finished as not to interfere with the time/duration and the effects of the sedation, dark adaptation, and mydriasis. The pupil diameter after the second ERG recordings were 4.46 ± 0.35 mm. Signs of systemic toxicity typical of those from the application of neuromuscular blocking agent such as neck and limb muscle paralysis were not observed from the topical application of rocuronium bromide.

In the scotopic mixed rod and cone response and the photopic cone response, the ERG of the domestic pigeons showed a negative deflection followed by a positive ascending slope, which were identified as the a- and b-waves, respectively (Fig. 1A–D). The photopic flicker introduced at a stimulus intensity of 3.0 cd sec/m^2, and a frequency of 27.78 Hz showed a series of waves following each light stimulus (Fig. 1E and 1F).

During the scotopic mixed rod and cone response, the implicit times obtained during the ERG of the control group were 11.67 ± 2.17 and 38.15 ± 9.35 msec for the a- and b-waves, respectively. With the induction of mydriasis on the treatment group, the implicit time for the a- and b-waves were 12.82 ± 0.64 and 31.92 ± 2.60 msec, respectively. The ERG amplitudes obtained in the control group for the a- and b-waves were 46.80 ± 25.93 and 145.52 ± 56.92 µV compared to 100.30 ± 62.13 and 206.81 ± 132.41 µV in the treatment group, respectively (Table 1). There were no statistically significant differences of the a- and b-wave implicit times and amplitudes in the scotopic mixed rod and cone ERG of the domestic pigeons between the control and the treatment groups.

However, in the photopic condition, statistically significant differences were observed in the a- and b-wave amplitudes of the photopic cone response in which the amplitudes of both the a- and b-waves were higher in the ERG of the treatment group (*P*=0.045 and *P*=0.032, respectively). The a- and b-waves of the photopic cone ERG amplitudes in the treatment group were 63.83 ± 32.33 and 191.75 ± 94.46 µV, which were significantly higher than 46.15 ± 27.60 and 116.76 ± 70.65 µV in the control group, respectively (Table 2). In the photopic flicker response, the flicker amplitude was also significantly higher in the treatment group (76.23 ± 48.56 µV) than the control group (42.18 ± 31.18 µV; *P*=0.044; Table 3). As for the implicit times, there were no

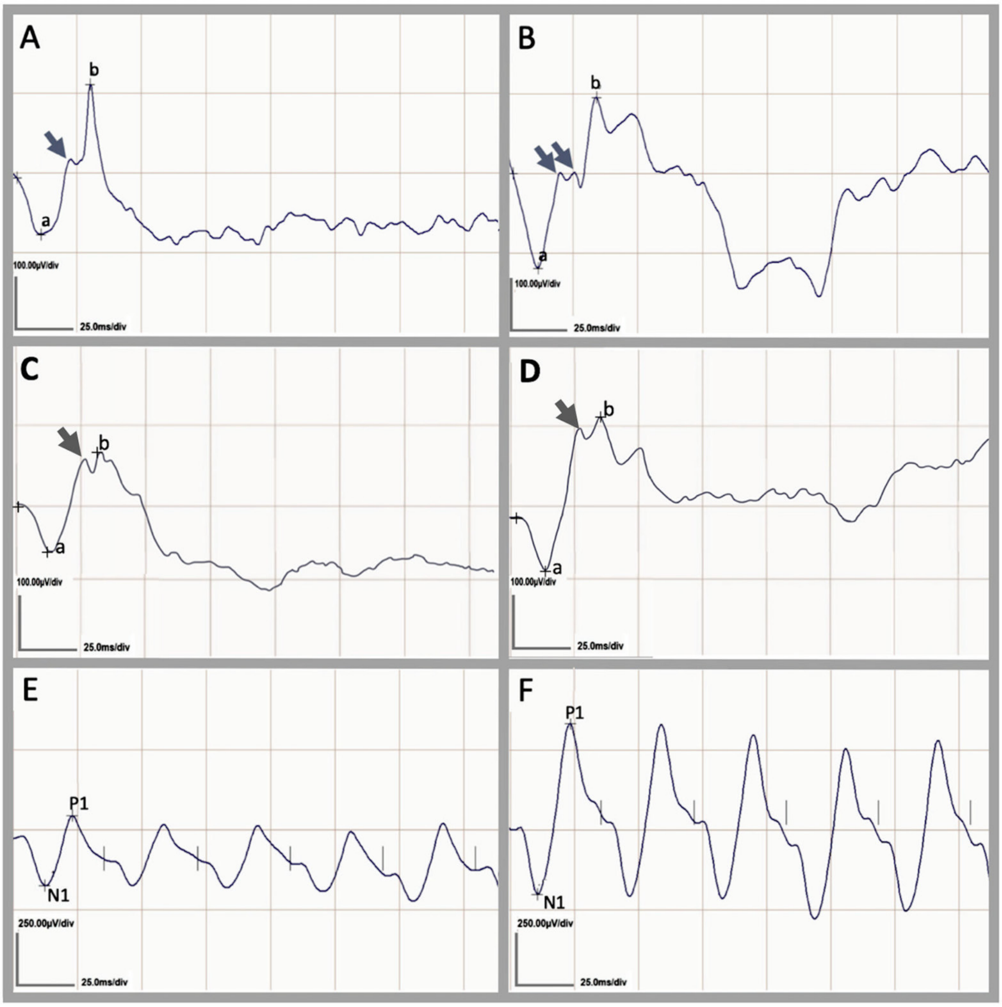

Fig. 1. Representative electroretinography of domestic pigeons with and without rocuronium bromide to induce mydriasis. The scotopic mixed rod and cone response: **(A)** without rocuronium bromide, and **(B)** with rocuronium bromide to induce mydriasis. The photopic cone response: **(C)** without rocuronium bromide, and **(D)** with rocuronium bromide. The photopic flicker response: **(E)** without rocuronium bromide, and **(E)** with rocuronium bromide. The letters a and b denote the a- and b-waves, respectively. N1 denotes the implicit time, and P1 denotes the amplitude of the photopic flicker response. The bar in between the waves of photopic flicker represents the introduction of light stimulus except for the first stimulus (on E and F). Oscillatory potentials (OP) were observed during on the ascending slope between the a- and b-waves (arrows; A, B, C and D).

statistically significant differences between the two groups in both the photopic cone and photopic flicker responses. The implicit times of the a- and b-waves obtained during the photopic cone ERG in the control group were 11.60 ± 2.07 and 30.37 ± 6.32 msec compared to 10.98 ± 0.73 and 27.55 ± 3.23 msec in the treatment group, respectively (Table 2). The implicit time for photopic flicker in the control group was 12.28 ± 1.63 msec compared to 11.95 ± 1.58 msec in the treatment group (Table 3).

Oscillatory potentials (OPs) were observed in the ascending slope between the a- and b-waves of the scotopic mixed rod and cone response and photopic cone response (Fig. 1A–D). These oscillatory potentials were composed of up to three peaks in this study.

DISCUSSION

ERG is generated by the summed activity of all retinal cells that consist of overlapping positive and negative component potentials originated from different stages of retinal processing [10]. The initial negative wave in response to light stimuli is known as the a-wave, followed by the positive peak known as the b-wave [7, 10, 22] (Fig. 1). Under the scotopic condition, the main generator of the a-wave to a low intensity stimulus is the rod photoreceptors, as the cone photoreceptors are not as sensitive to light [22]. However, in this study, the stimulus presented during the scotopic condition was strong enough to elicit a response from both photoreceptor types (Fig. 1A and 1B). This protocol was based on the ERG guideline for dogs in which the bright stimulus of 3.0 cd sec/m^2 was designed to elicit rod and cone responses after the animal underwent a dark adaptation stage [8]. As for the photopic condition, the a-wave was driven by cone photoreceptors because the rod photoreceptor was suppressed by the light adaptation stage (Fig. 1C and 1D) [22]. Aside from the photoreceptors, the bipolar cells, amacrine, and ganglion cells also contribute to the

Table 1. Descriptive statistics of the scotopic mixed rod and cone electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Group	Scotopic mixed rod and cone response			
	Implicit time (msec)		Amplitude (µV)	
	a-wave	b-wave	a-wave	b-wave
Control	11.67 ± 2.17*	38.15 ± 9.35	46.80 ± 25.93	145.52 ± 56.92
Treatment	12.82 ± 0.64	31.92 ± 2.60	100.30 ± 62.13	206.81 ± 132.41

*Mean ± standard deviation.

Table 2. Descriptive statistics of photopic cone electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Group	Photopic cone response			
	Implicit time (msec)		Amplitude (µV)	
	a-wave	b-wave	a-wave	b-wave
Control	11.60 ± 2.07*	30.37 ± 6.32	46.15 ± 27.60[a]	116.76 ± 70.65[a]
Treatment	10.98 ± 0.73	27.55 ± 3.23	63.83 ± 32.33[b]	191.75 ± 94.46[b]

Wilcoxon rank-sum test (Mann-Whitney *U* test) was used to compare the a-wave implicit times between control and treatment groups. The b-wave implicit time and the a- and b-wave amplitudes were analyzed using Student's *t*-test. [a,b] Different superscript letters indicate significant differences in the same column at a *P*-value of <0.05. *Mean ± standard deviation.

Table 3. Descriptive statistics of photopic flicker electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Group	Photopic flicker response	
	Implicit time (msec)	Amplitude (µV)
Control	12.28 ± 1.63*	42.18 ± 31.18[a]
Treatment	11.95 ± 1.58	76.23 ± 48.56[b]

Student's *t*-test were used to compared the implicit times and amplitudes between the control and treatment groups. [a,b] Different superscript letters indicate significant differences in the same column at a *P*-value of <0.05. *Mean ± standard deviation.

a-wave [10, 22]. In both dark- and light-adapted conditions, the main generator of the b-wave is the ON-bipolar cells [10].

During ERG, the contribution from each retinal cell generally increases with the area of the retina stimulated as the number of cells, and hence the total extracellular current is increased [10]. Mydriasis allows more photons to enter the eye, resulting in increased retinal illumination [21]. Therefore, it could be expected that ERG recorded in a mydriatic pupil (treatment group) would result in a higher value as seen in both the a- and b-wave amplitudes of the photopic cone and photopic flicker response in this study (Tables 2 and 3). Performing ERG on miotic pupils, not only could potentially reduce the amplitude, but might also increase the implicit time [7]. This lower amplitude and attenuated implicit time might be mistaken for retinal disease, which was why ERG was recommended to be performed on eyes with mydriatic pupils [7]. In the present study, no differences were observed between the amplitudes of the mydriatic and non-mydriatic pupils on the scotopic condition and only a lower amplitude was observed in the photopic ERG of the control group compared to the treatment group (in both photopic cone and photopic flicker response). Furthermore, no differences were observed in the implicit time from all ERG recordings.

The lack of a significant difference between both groups in the implicit times and amplitudes of our scotopic results might be caused by the possibility that in the dark-adapted stage, the pigeon's pupil underwent mydriasis naturally. Therefore, the scotopic ERG between both groups was not significantly different as both pupils were dilated. In contrast, the photopic protocols were preceded by a light adaptation stage in which the pigeon's eye was illuminated for 10 min with a background light of 30 cd/m^2. This light adaptation procedure was designed to suppress the function of rod photoreceptors but, at the same time, would also constrict the pupil in the control group in the absence of rocuronium bromide to keep the pupil dilated. Study in human comparing ERG between eyes with dilated pupil and non-dilated pupil observed that in scotopic condition the non-dilated pupil underwent some degree of natural dilation but never achieved the diameter resulted from the application of mydriatics [11]. The human study also showed significant retinal sensitivity reduction in eye without mydriatics [11]. Differed with the present study, the b-wave of the scotopic ERG in human showed significant different between the non-dilated and dilated eye [11]. However, upon eliminating one seemingly misplaced data, the different between both conditions became insignificant [11] which in agreement with the present study.

In human, pupil dilates significantly less in elderly compared to the young [11]. ERG study in pigeon with a wide range of age reported no systematic differences in pupil diameter between the old and young pigeon [24]. The mydriatic agent used in the present study was rocuronium bromide, a neuromuscular blocking agent with an affinity for acetylcholine (ACh) receptors, which acts as an ACh competitor in the neuromuscular junction of the bird's iris striated muscle, resulting in mydriasis [13]. Previous ERG study in pigeon utilized topical d-tubocurarine chloride to induce mydriasis [1, 24] and although d-tubocurarine chloride was reported as unreliable in inducing mydriasis [28], the pupil diameter achieved in the previous study was 4.6−5 mm in diameter [24]. In the present study, the degree of mydriasis achieved by rocuronium bromide was only measured after the ERG recording had ended. This was done to avoid the delay on ERG recording and therefore waste the effect of sedation and mydriasis as well as potentially disrupt the dark-adapted retina. The pupil diameter after the ERG procedure ranged from 4.00 to 5.00 (4.46 ± 0.35) mm. Since mydriasis produced by rocuronium bromide in pigeon was observed after 5 min and lasting for two hours [27], the mydriatic effect after 20 min of dark adaptation should be optimal to facilitate ERG recording.

The current guideline for ERG in dogs advocates 20 min of dark adaptation stage before the scotopic mixed rod and cone response to allow for the rod photoreceptor to gain its sensitivity [8]. The dark adaptation stage employed on the previous report of ERG performed on birds had been varied from 4 min [12], 5 min [25], 10 min [15] and 20 min [16, 26]. However, since the protocol and settings of ERG performed were all different, it was hard to compare the results for all the study. In the present study, the dark adaptation was done for a minimum of 20 min before each scotopic ERG recording as suggested by the current ERG guideline [8]. In the comprehensive ERG testing, on the protocol for both dogs and human, a special test designed to evaluate rod function is performed during the dark adaptation stage called the rod-driven test [8]. In this test, a series of flashes with a low intensity is introduced every 5 min during at least 20 min of dark adaptation and (ideally) performed until the b-wave amplitude produced reaches a stable plateau [8]. However, this rod-driven response was not performed in this study as this test would require a prolong handling of the pigeon during the dark adaptation.

As discussed above, the main reason for mydriatic induction during ERG was to ensure equal distribution of the light stimulus on the retina in order to obtain a maximum amplitude and avoid prolonged implicit time [7]. However, study in mouse anesthetized with ketamine and xylazine, showed that certain combination of mydriatics could also directly affecting the electrogenesis on the retina [19]. The combination of atropine and phenylephrine was reported to produce a significantly higher b-wave amplitude when compared to atropine alone or without the use of mydriatics in ketamine and xylazine anesthetized mouse despite the similar pupil size [19]. The atropine and phenylephrine combination were thought to affect the signaling of the amacrine and ganglion cell circuit in the retina resulting in a higher b-wave amplitude [19]. Considering that the amacrine cell of the pigeon's retina also possessed ACh receptors [30] and rocuronium bromide binds to the ACh receptors, further study is needed to determine if rocuronium bromide could directly affect the electrogenesis on the pigeon's retina.

The a-wave and b-wave are the two waves most commonly used to evaluate retinal diseases in clinical setting [9]. It is well-known that several technical aspects can influence the amplitudes and implicit times of the a- and b-waves of the ERG recording, for example, sedation and anesthesia [7–9]. However, these effects are not always observed in pairs. For example, medetomidine and its isomer, dexmedetomidine, have consistently been reported to prolong the implicit time of both a- and b-waves and also lower its amplitudes when compared to awake dogs [9, 20]. In contrast, general anesthesia was found to lower both a- and b-wave amplitudes but only prolong the a-wave implicit time [9]. A comparison of alfaxalone and medetomidine sedation during ERG recording of pigeons also only differed in the a-wave implicit time and b-wave amplitude [26]. In the present study, although the non-mydriatic pupils of the control group resulted in lower amplitudes of both photopic cone and photopic flicker, none of the implicit times were prolonged.

Alfaxalone was used to induce sedation in this study as it was reported to have less effect on pigeon's ERG compared with alpha-2 adrenergic receptor agonists [26]. Here, the level of sedation obtained was adequate to facilitate the handling of pigeons throughout the procedure with minimal stress, but there is an inherit possibility that should alfaxalone has any suppression effects to the ERG recording, the early recording might have had been affected more by the sedation compared to the later. However, previous report on pigeons showed the b-wave of the photopic ERG recorded under alfaxalone sedation to be higher than that of medetomidine sedations [26], the later widely known to depress the ERG recordings [9]. The use of anesthesia during ERG recording has always been recommended as it will help to minimize the noise and artifacts from involuntary muscles, especially for a long ERG session [8, 9]. As for a shorter ERG protocols, performing ERG without chemical restrain is considered acceptable, with no significant differences in the low- or high-frequency noise levels found between sedated and anesthetized dogs [9]. ERG recordings of Hispaniolan Amazon parrots (*Amazona ventralis*) and two species of owl were performed under general anesthesia using isoflurane [12, 25], and sevoflurane was used for ERG recording in bald eagle (*Haliaeetus leucocephalus*) [15]. While mydriasis was not induced in those studies, isoflurane was known to cause mydriasis in birds [18], which might be beneficial in retinal illumination when mydriasis is not utilized during ERG recording.

In the present study, OPs ranged from one to three peaks at the ascending slopes between the a- and b-waves were observed in both pupil conditions. OPs are a series of wavelets superimposed mainly on the ascending limb of the b-wave whose number and appearance vary between individuals and species [7, 12, 29]. Three to five peaks are usually observed in the dark-adapted response in cats and dogs [7]. In Hispaniolan Amazon parrots, OPs were consisted of three to four peaks [12]. Although special adaptation levels and filtering techniques are required to record OPs, sometimes they are seen during the recoding of a- and b-waves [29]. In this study, photopic flicker was introduced at a frequency of 27.78 Hz even though pigeons have the highest flicker-fusion frequency reported among vertebrates, of 143 Hz [17]. Performing photopic flicker ERG in non-mydriatic pupils (control group) resulted in a lower amplitude than photopic flicker in mydriatic pupils (treatment group).

In conclusion, rocuronium bromide provide an adequate mydriasis on pigeon underwent ERG resulting in higher amplitudes of both photopic cone and photopic flicker ERG. However, as pigeon pupil naturally underwent mydriasis in scotopic condition, there were no significant different observed during the scotopic mixed rod and cone ERG.

CONFLICT OF INTEREST. The authors declared no conflict of interest.

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