http://dx.doi.org/10.5380/avs.v1i1

Archives of Veterinary Science
https://revistas.ufpr.br/veterinary

The effect of eye stimulation using graded external weights on the oculocardiac reflex in beagle dogs

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ARTICLE INFO

Keywords:
autonomic nervous system;
heart rate variability;
ocular compression;
vagus nerve;
von Frey

Received: 10/05/22
Accepted: 30/08/22
Published: 01/03/23

ABSTRACT

The objective of this study was to determine to what extent the oculocardiac reflex (OCR) progressively rises with increasing external pressures over the superior eyelid. Controlled external weights were applied to the left eyelid of ten conscious healthy adult beagle dogs, using an electronic von Frey aesthesiometer with a modified probe. Four different weights (200 g, 300 g, 400 g and 800 g) were applied on four different non-consecutive days (with one-week intervals). Pressure was applied by pushing the device against the superior eyelid. The baseline heart rate was primarily recorded using an electrocardiograph (ECG). Then, indices of heart rate variability such as R-R intervals and vasovagal tonus index (VVTI) were calculated from the ECG tracings using twenty consecutive QRS complexes from each dog, before and after a selected set of graded weights were applied to the eye. Median R-R interval significantly increased when an external weight of 200 g was applied and peaked at 300 g. VVTI values peaked at 400 g. With higher pressures, the proportional increase was smaller in both R-R intervals and VVTI. We concluded that external eye pressure using a weight of 200 g or more is sufficient to elicit an OCR response in conscious dogs. Pressures above 400 g started to activate compensatory mechanisms that counteracted with the OCR, probably linked to an adrenergic response induced by the animal's discomfort. Changes in R-R intervals might be used as an early OCR marker, while VVTI apparently was less influenced by potential sympathetic responses.

1. Introduction

The oculocardiac reflex (OCR) is a physiological response of the heart following mechanical stimulation of the ocular globe or adnexa manifested as bradycardia of ≥10% decrease in heart rate (HR) (Aschner, 1908). The OCR can be life threatening and may occur in complex forms with arrhythmias, atrioventricular blocks, ventricular fibrillation, and even asystole (Dunville et al., 2021; Karaman et al., 2015). Oculocardiac reflex is well documented in human patients with facial trauma and fractures (Fahling e Mckenzie, 2016; Borumandi et al., 2014), orbital tumor (Rosa, 2017), subconjunctival injections (Kayikçioglu et al., 1999), and ocular surgeries (especially pediatric surgeries for strabismus correction) (Choi et al., 2009; Dunville et al., 2021; Waldschmidt and Noah, 2019). In dogs, OCR was described in patients with choroidal melanoma extending to the orbit (Steinmetz et al., 2012), as well as with zygomatic fracture (Ghaffari et al., 2009). Additionally, experimentally induced OCR was well-characterized in anesthetized (Clutton et al., 1988; Gandevia et al., 1974) and conscious dogs (Giannico et al., 2014).

The effect of manual pressure on one or both eyes on the OCR in dogs was previously studied by our group (Giannico et al., 2014). However, in our previous investigation, the magnitude of manual pressure applied was not measured. The electronic von Frey aesthesiometer was designed to perform quantitative sensory testing by measuring the pressing force applied on a given tissue (Cunha et al., 2004; Sato et al., 1976).

The heart does not work as a continuous pump; each heartbeat is a unique event, influenced alternatively by sympathetic or parasympathetic autonomic nervous systems (Doxey and Boswood, 2004; Moonamart et al., 2012; Little et al., 1999). Heart rate variability (HRV) is a non-invasive technique to assess autonomic nervous system function and is classified in two major branches: frequency and time domain. Vasovagal tonus index (VVTI) is a time-domain analysis calculated by the natural logarithm of the variance from normal R-R intervals (i.e., the interval between heart beats) in the electrocardiogram (Moonamart et al., 2012) The VVTI provides information about variations in heart rate elicited from fast vagal activation and has been described in healthy conscious dogs. It its widely used for sympathovagal balance studies as a diagnostic and prognostic tool for heart failure in dogs (Brüler et al., 2018; López-Alvarez et al., 2014; Martinez Pereira et al., 2008).

The purpose of this study was to investigate the correlation between the amount of weight applied onto the canine eye and the OCR by means of the electronic von Frey aesthesiometer with a modified non-traumatic rubber probe. Our hypothesis was that OCR would progressively increase with increasing external pressure.

2. Material e Methods

Ten (five females and five males) healthy one-year-old beagle dogs (Canis familiaris) from a canine colony of an animal nutrition laboratory located at the Federal University of Paraná (UFPR), Brazil were used in this study. Dogs were cared for and handled according to the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in vision and ophthalmic research. All dogs were housed in similar conditions and underwent a complete ophthalmic and general physical examination prior to enrollment, all being classified as healthy and free of ophthalmic and systemic disorders. No dogs were under any type of medication during the time of enrollment. The study protocol was approved by the local university´s ethics committee.

The study was performed in four non-consecutive days at approximately the same time of day to avoid circadian variations. In addition, the timing chosen was of at least two hours after the last meal, as feeding can influence autonomic tone. Dogs were positioned in a right lateral recumbency and maintained in this position by gentle physical restrain. A few minutes were given for dogs to acclimatize and calm down. Then, electrodes connected to an electrocardiograph (ECG) machine (ECGPC-TEB, Brazilian Electronic Technology Ltda., São Paulo, SP, Brazil) were attached to the skin of the animals and wet with isopropyl alcohol (70%) to improve electrical conduction. The electrodes used were smooth alligator-types to minimize discomfort. The left and right arm electrodes were placed at the elbows, while the left and right leg electrodes were placed at the stifles.

ECG was performed continuously and uninterrupted for three minutes, at a speed of 25 mm/s.

During the whole last minute, external ocular pressure was manually applied to the left eye using an electronic von Frey aesthesiometer (EFF 301, Insight, São Paulo - Brazil). The original device utilizes interchangeable disposable, rigid standard pipet tips made of polypropylene with the length of 71mm to perform sensory tests. In our study, we used a modified 2.4 x 2.4 x 2.4 cm styrene-butadiene rubber probe (Figure 1) weighting 25 g, which was designed to increase the surface area of the tip. This allows even distribution of the force applied onto the globe while preventing trauma to the eye. The weight of the modified probe was electronically adjusted (tared) to zero.

Mechanical pressure was delivered by manually pushing the rubber probe against the eye over the superior eyelid, always ensuring no contact was made between the device and the surrounding bony orbit. The eye was externally pressured using four different constant weights (200, 300, 400, and 800 g) for a full minute each. A conscious attempt was made in order to carefully place the rubber probe always at the center of the left superior eyelid, making sure the weight was being applied in the vertical axis (perpendicular to the testing site). In each animal, the baseline HR was recorded initially for one minute, followed by one minute of pressure. The pressures were applied in four different non-consecutive days (at one-week intervals) in the same animals, interchanging different weights in the following order 200, 800, 300, and 400 g each day.

For statistical purposes, the first twenty consecutive R-R intervals of sinus origin after the end of the first minute and the first 20 intervals after 10 seconds of ocular compression were measured on the electrocardiogram, by an author blinded to the study conditions. Average heart rate and VVTI before and after OCR were obtained. VVTI was calculated as follows: VVTI = LN [VAR (R-R1- R-R20)], where LN is natural logarithm, and VAR is the variance. The resulting VVTI is dimensionless. Data was tested for normality using the Shapiro-Wilk test. HR data followed a normal distribution. Therefore, paired t-tests were used to compare HR data before and after each compression. R-R interval and VVTI data did not present normal distributions. Consequently, Kruskall-Wallis tests followed by Dunn's post hoc tests were used to compare the R-R intervals and VVTI obtained with all different ocular external pressures. P-values were considered significant at $P<0.05$. Software Sigma XL version 7.0 (Kitchener, Canada) was used in all statistical analysis.

3. Results

Overall, all dogs were very calm during the study and tolerated well every one of the different weights applied, with the exception of two dogs that showed slight discomfort (i.e. becoming somewhat more agitated and a bit reluctant to stay in lateral recumbence) in response to the heaviest weight. This fact marks an important limitation of this study when transporting the results to anesthetized animals and will be discussed further on.

The average heart rates, the median R-R intervals and the median VVTIs obtained at each of the different weights applied are listed in Table 1, Figure 2 and Table 2, respectively.

There was a statistically significant increase in R-R intervals (resulting in a decreased HR) recorded during the application of all different external pressures when compared to baseline ($P<0.0001$).

Median R-R interval proportionally increased 10.83% (meaning higher values were obtained with the eye under pressure when compared to baseline values) when 200 g was applied and peaked with the external weight of 300 g (a 20.11% increase). The median R-R interval still increased when 400 g and 800 g of external weight were used (9.4 and 13.39%, respectively). Both increased proportionally less than when a 300 g stimulus was applied.

Figure 1 *– A close-up view of the modified rubber probe of the electronic von Frey aesthesiometer device.*

Baseline Heart Rate (bpm)	External weight (g)	Heart rate during ocular compression (bpm)	P-value
111.53 ± 17.29	200	99.93 ± 19.30	<0.0001
111.78 ± 22.57	300	95.18 ± 23.44	<0.0001
130.64 ± 13.06	400	117.04 ± 21.36	<0.0001
147.53 ± 20.53	800	128.77 ± 20.78	<0.0001

Table 1 *– Average heart rates in beats per minute (bpm) before and after left eye compression using different external weights.*

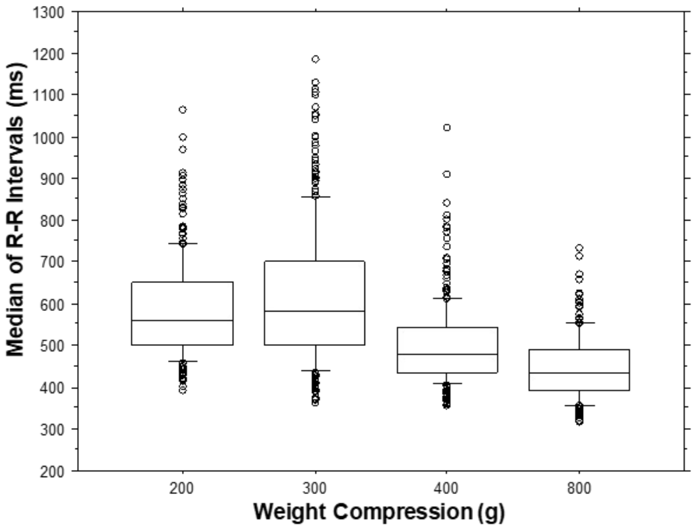

Figure 2 *– Box-Plot graph demonstrating median baseline R-R intervals and R-R values after ocular compression using different external weights. Note that R-R intervals increase in parallel when increasing external weights up to 300 g are used. With heavier stimulus, the corresponding proportional increases in R-R intervals are of a smaller magnitude.*

Baseline Heart Rate (bpm)	External weight (g)	Heart rate during ocular compression (bpm)	P-value
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147.53 ± 20.53	800	128.77 ± 20.78	<0.0001

Table 2 *– Baseline representing median vasovagal tonus index (VVTI) and interquartile range (IQR) before and after each different external pressure was applied on the left eye using different external weights.*

A statistically significant increase in median VVTI values was noted in response to all external pressures ($P \leq 0.037$) except when the weight of 200 g was applied ($P=0.13$). Median VVTI values were higher (5.7% rise using 200 g and 5.54% using 300 g) compared to baseline values. Noticeably, a weight of 400 g proportionally caused a slightly greater VVTI increase (a 15.06% rise) when compared to 800 g (13.98% rise).

4. Discussion

This study aimed at determining to what extent the OCR progressively rises with increasing external ocular pressures in conscious dogs.

It is worth mentioning that the median of baseline R-R intervals and VVTI slowly but progressively decreased (resulting in increased HR) at each given test using increasing weights (Tables 1 and 2), possibly because the dogs became progressively more anxious, anticipating the test experienced the week before. Thus, the possibility of the existence of an interference of psychological origin during the baseline measurements cannot be entirely ruled out, this being a study limitation. However, since each dog served as its own (paired) control (before and after the weight was applied), the proportional change could still be analyzed, regardless of the initial individual level of pre-test excitement. The present study started with a minimum external stimulus of 200 g, which proved to be sufficiently reflexogenic. Smaller external pressures were not investigated. This, however, could be investigated in the future in order to characterize the minimum weight necessary to elicit an OCR in the dog (threshold).

Even the lightest external weight applied in our study (200 g) was sufficient to elicit OCR in conscious dogs, considering the increase in R-R intervals. An external stimulus of 200 g triggered a significant reduction in HR, but not enough to significantly change the VVT, conceivably meaning that R-R intervals can be used as an early OCR marker. When using an external weight of 300 g, a more expressive increase in R-R intervals was induced and, therefore, the resulting proportional reduction in HR was even more prominent. The response to 300 g led to a greater variability in HR and consequently a significant increase in VVTI. For the next two heavier weights applied (400 and 800 g), the values of the R-R intervals still increased significantly, but proportionally less than the previous ones (Table 1 and Figure 2), probably because the parasympathetic stimulus resulting from external pressures was masked by a sympathetic stimulus. Moonamart et al. (2012), Doxey and Boswood (2004) Trauffler et al. (2019) found that the values of VVTI in healthy dogs are of approximately 8, similar to what was seen in initial baseline values in this study. It took a heavier external weight (800 g) for this specific marker to start to proportionally drop, while R-R intervals started to increase as soon as 400 g of external weight was used. Thus, VVTI seemingly showed to be somewhat less influenced by a potential sympathetic response caused by stress. Moonamart et al. (2012), has previously shown that excitation during clinical examination does not affect the VVTI measurements in healthy dogs.

A possible physiological explanation for the results of our study would be the existence of an intrinsic sympathetic compensatory mechanism responding to the external pressures applied to the globe, which counteracts and limits the OCR. The existence of a sympathetic component to the OCR has been previously suggested by Paton et al. (2005). Another possible explanation would be that, when heavier external weights, i.e., 400 and 800 g were used, the resulting ocular discomfort (even if discrete) activated the nociception system with a subsequent adrenergic activation (Paton et al., 2005) leading to a proportionally smaller OCR response.

It is known that the type of mechanical stimulus on the eye influences the magnitude of OCR (Khurana et al., 2006). Binocular external pressures applied to the eyes deliver more profound OCR in comparison to monocular (Joffe and Gay, 1966; Gandevia et al., 1978). We have applied acute mechanical pressure (and release) using a weight of 200 g on one globe and found a significant increase in R-R intervals. Nevertheless, a binocular stimulus might have evoked an even greater OCR.

In anesthetized animals, the pain threshold would be expectedly higher, so the effect of ocular pressure weights should likely be smaller than in conscious animals. In fact, the deeper the anesthetic plane, the less likely an OCR might occur (Arnold, 2021a). Thus, we cannot rule out the possibility that, in the absence of pain or discomfort (i.e. general anesthesia or nerve block), the OCR would continuously increase with the higher weights used in our investigation. On the other hand, different anesthetic drugs might also affect the OCR by having inhibiting or exacerbating effects. For instance, inhalation anesthetic drugs might have different degrees of vagolytic action in dogs: desflurane has shown to have the highest vagolytic effect, followed by sevoflurane, isoflurane, enflurane and, finally, halothane (Picker et al., 2001). The increasing doses of sufentanil proportionally decrease heart rate in dogs (Carareto et al., 2007). In humans, sevoflurane and desflurane are safe to use in strabismus surgery in pediatric patients (Oh et al., 2007). Opioids (Arnold et al., 2021b), propofol (Smith et al., 1994), dexmedetomidine and apparently dexamethasone may cause bradycardia and can augment OCR (Arnold et al., 2021b). Additionally, preoperative administration of anticholinergic drugs as atropine and glycopyrrolate in dogs may not prevent OCR occurrence during enucleation (Vézina-Audette, 2019). Only opioid induced bradycardia responds to anticholinergic drugs. The retrobulbar nerve block with bupivacaine and lidocaine was associated with lower OCR in dogs during enucleations (Vézina-Audette, 2019). Carefully drug volume should be chosen in retrobulbar block considering its occasionally OCR induction (Park et al., 2009).

In human patients, ketamine showed inhibitory effect in parasympathetic receptors (Espahbodi et al., 2015). Although there is an active search for a better technique or anesthetic drug to decrease OCR in human patients, the autonomic balance is similar but not identical in dogs and humans (Picker et al., 2001), and studies targeting this goal in veterinary patients are scarce. Finally, although this study was performed in conscious dogs, therefore a role of the sympathetic tone due to discomfort counteracting with the OCR cannot be ruled out, we believe the results obtained may shed a light in how different weights applied to the ocular surface affect the OCR during surgeries in anesthetized patients.

5. Conclusion

This study is the first to characterize canine OCR by graded stimuli using measured ocular weights. It shows that the OCR is elicited in monocular weight stimulus of as little as 200 g in conscious dogs and its intensity is force-dependent up to a certain point, and then it starts to suffer interferences, possibly from the nociception system and subsequent adrenergic stimulation.

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