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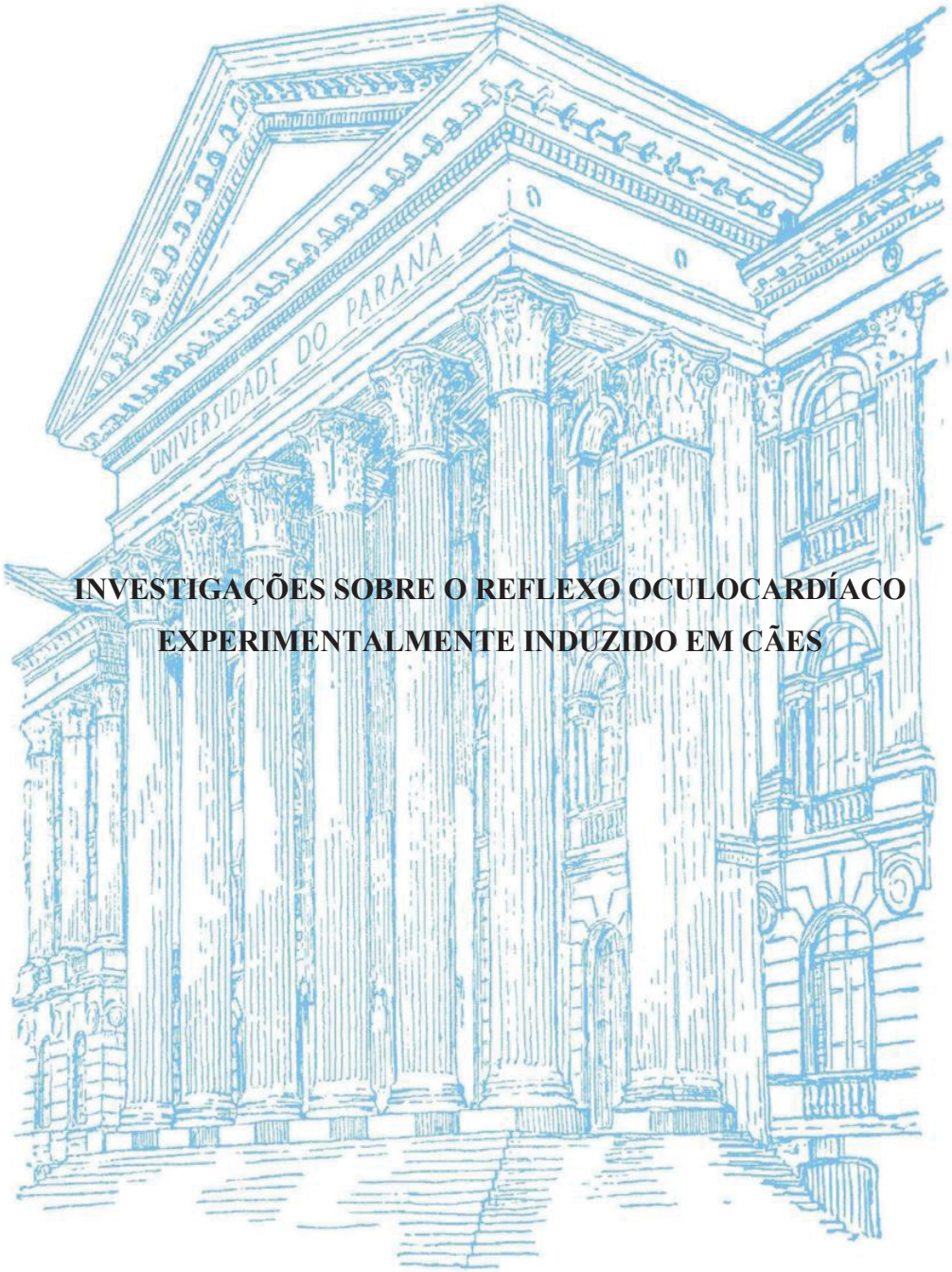

INVESTIGAÇÕES SOBRE O REFLEXO OCULOCARDÍACO EXPERIMENTALMENTE INDUZIDO EM CÃES

CURITIBA
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná como pré requisito parcial para a obtenção do título de Mestre.
Orientador: Prof. Dr. Fabiano Montiani-Ferreira

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

UFPR

PARECER

A Comissão Examinadora da Defesa da Dissertação intitulada **"INVESTIGAÇÕES SOBRE O REFLEXO OCULOCARDÍACO EXPERIMENTALMENTE INDUZIDO EM CÃES"** apresentada pela Mestranda **THAYANE CRISTINE SANTOS VIEIRA** declara ante os méritos demonstrados pela Candidata, e de acordo com o Art. 79 da Resolução nº 65/09–CEPE/UFPR, que considerou a candidata APTA para receber o Título de Mestre em Ciências Veterinárias, na Área de Concentração em Ciências Veterinárias.

Curitiba, 29 de março de 2017

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“Toda a nossa ciência, comparada com a realidade, é primitiva e infantil-, e, no entanto, é a coisa mais preciosa que temos” (Albert Einstein)

Agradecimentos

Ao meu admirado orientador Prof. Fabiano Montiani-Ferreira pela oportunidade, confiança, paciência e ensinamentos.

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À minha amada Brida.

RESUMO

Esta pesquisa buscou aprofundar a caracterização do reflexo oculocardíaco em cães (ROC, ou *oculocardiac reflex- OCR* em inglês) até então pouco descrito em literatura e pesquisas. Denomina-se ROC a resposta neurofisiológica à compressão do bulbo ocular ou manipulação das estruturas próximas e associadas (como músculos extrao-oculares e órbita). Este reflexo pode desencadear alterações graves relacionadas à frequência e ao ritmo cardíaco, inclusive parada cardíaca. O ROC é frequentemente descrito em procedimentos cirúrgicos oftálmicos em seres humanos, no entanto em cães existe pouca documentação tanto da prática clínica quanto da indução experimental. Ainda não há documentação do ROC envolvendo cães que receberam diferentes drogas anestésicas nem tampouco empregando eletrocardiograma. Em seres humanos a diminuição de 10% na frequência cardíaca durante a manipulação do bulbo ocular ou estruturas peri-oculares atribui-se ao aparecimento do ROC. Em vista desses fatos, este estudo foi dividido em dois capítulos independentes: o primeiro tem o intuito de descrever se existe um sinergismo entre o ROC e a associação de fármacos anestésicos mais utilizados na Medicina Veterinária (acepromazina e morfina ou petidina), já o segundo procura verificar se o ROC apresenta expressão força-dependente através do analgesímetro eletrônico de von Frey para determinar as forças aplicadas. Ambos os capítulos usaram como análise um método até então usado com mais frequência na cardiologia veterinária: a variabilidade da frequência cardíaca (VFC). Os achados são interessantes por não se tratarem de resultados esperados em ambos os estudos. Ressalta-se que ainda não existe um método ouro para minimização ou prevenção do ROC e suas possíveis consequências, sendo de suma importância sua investigação detalhada.

Palavras-chave: sistema nervosa autônomo, parassimpático, nervo vago, tônus vagal, variabilidade da frequência cardíaca, medicação pre-anestésica, petidina, von Frey, cão

ABSTRACT

The purpose of this research was to improve the knowledge of oculocardiac reflex (OCR) in dogs, so far slightly described in literature and previous papers. OCR is a neurophysiological response in stimulation of the eye itself or around structures (extra-ocular muscles, orbit). This reflex might lead serious heart rate and rhythm disturbances (even an asystole). In humans beings usually is described in ophthalmic surgeries, consisting in a sudden onset bradycardia of 10% or more in heart rate comparing to baseline. Otherwise, there is no documentation of OCR involving dog patients with different anesthetic drugs nor using de electrocardiogram. In dogs, none parameter was found to determine of occurrence of OCR or the mechanic pressing needs to trigger it. There is a little registered material in literature or experimentally on this field in Veterinary Medicine. Hence, this research was divided into two independent chapters: the first one with the aim to describe the hypothesis of existing synergism between the OCR and the association of drugs most used in Veterinary Medicine (acepromazine and morphine or pethidine), and the second, discusses if the OCR has a force-dependent component by using electronic von Frey anesthesiometer to measure forces applied. Both chapters used to analysis Cardiology non-invasive method: heart rate variability (HRV). A gold standard method or drug to prevent OCR was not found yet. These promissory and unexpected findings are significantly to better understanding and minimizing the OCR, in face of its possible consequences.

Key-words: autonomic nervous system, parasympathetic, vagus nerve, vagal tonus, heart rate variability, pre anesthethic medication, pethidine, von Frey, dog

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LISTA DE ABREVIATURAS E SIGLAS

ITVV	índice de tônus vasovagal
LENUCAN	Laboratório de Estudos em Nutrição Canina
ROC	reflexo oculocardíaco
VFC	variabilidade da frequência cardíaca

ABBREVIATIONS LIST

AMG	acepromazine + morphine group
APG	acepromazine + pethidine group
APM	after preanesthetic medication
ASA	American Society of Anesthesiologists
BPM	before preanesthetic medication
HRV	heart rate variability
MRR	mean of R-R intervals
OCR	oculocardiaco reflex
OD	oculus dexter
OS	oculus sinister
OU	oculus uterque
rMSSD	root mean square of sucessive R-R intervals differences
SD	standard deviation
SE	standard error
SDNN	standard deviation of normal-to-normal intervals
VVTI	vasovagus tonus index

SUMÁRIO

CAPÍTULO 1

Effects of premedication with acepromazine, morphine and pethidine on the experimentally induced oculocardiac reflex in dogs

Resumo

O propósito deste trabalho foi de determinar os possíveis efeitos de drogas anestésicas usadas com maior frequência na medicação pré-anestésica sobre a intensidade do reflexo oculocardíaco (ROC) experimentalmente induzido em cães. Quartorze cães da raça Beagle clinicamente saudáveis foram divididos em dois grupos para receberem via intramucular a associação de duas drogas: 1) morfina (0,3 mg/kg) + acepromazina (0,03 mg/kg) (n=7) e 2) petidina (4mg/kg) + acepromazina (0,03 mg/kg) (n=7). Ambos os grupos foram submetidos a um protocolo de estímulo do ROC em dois momentos: 2 horas antes da medicação pré-anestésica (BPM) e 15 minutos após a medicação pré-anestésica (APM). O ROC foi provocado por compressão digital contínua aplicada ao bulbo ocular com o polegar acima da pálpebra superior. A compressão teve a duração de um minuto iniciando pelo olho direito (OD) de forma isolada, depois olho esquerdo (OS) e por fim ambos os olhos (OS). Um descanso de compressão ocular foi realizado entre cada compressão, momento em que permanecia apenas com contenção física. O eletrocardiograma monitorou todos os estágios. Para análise foi usada a variabilidade da frequência cardíaca (VFC), em milissegundos pela mensuração de vinte intervalos entre batimentos (intervalos de onda R). Na VFC foram calculados no domínio do tempo: média intervalos R-R (MRR), o desvio padrão intervalos normais R-R (SDNN), a raiz quadrada da diferença entre entre intervalos entre onda R sucessivos (rMSSD) e o índice de tônus vasovagal (ITVV). A acepromazina associada a morfina ou a petidina tem efeito sinérgico com o ROC experimental em cães. De forma inesperada a acepromazina associada à petidina, apesar da estrutura química da última ser semelhante à dos fármacos anticolinérgicos, aumentou o intervalo entre ente picos de ondas R. Ou seja, causou maior efeito bradicárdico do que quando comparada a morfina + acepromazina, aprofundando o efeito do ROC. Tendo em vista do risco que o paciente de procedimento cirúrgico oftálmico pode ser submetido, é evidente considerar os efeitos das drogas sobre a intensidade do ROC.

Palavras-chave: sistema nervosa autônomo, variabilidade da frequência cardíaca, compressão ocular, medicação pré-anestésica, nervo vago, índice do tônus vasovagal

Abstract

Objective To determine the effects of preanesthetic medication with acepromazine associated with morphine or pethidine on the intensity of oculocardiac reflex (OCR) experimentally evoked in dogs.

Animal studied Fourteen clinically healthy seven-year-old Beagle dogs.

Procedure The dogs were allocated into two groups to receive two different preanesthetic medications intramuscularly: morphine (0.3 mg/kg) and acepromazine (0.03 mg/kg) (AMG, n = 7) or pethidine (4 mg/kg) and acepromazine (0.03 mg/kg) (APG, n =7). Both groups underwent a stimulation procedure divided in two moments: two hours before the administration of premedication (BPM) and fifteen minutes after premedication (APM). The OCR was stimulated by digital compression over the superior eyelid, first on the right eye (OD), then on the left (OS) and finally on both eyes (OU), all separated by one minute intervals. Between each compression period there was one-minute pause without manipulation of the eyes, summating seven stages. An electrocardiogram recorded heart rate in every stage. To evaluate the effects of the anesthetic protocols on the OCR, 20 R-R peak wave intervals were measured in milliseconds. In time domain of heart rate variability, the following variables were calculated to support data: the mean of R-R intervals (MRR), the standard deviation of the normal-to-normal interval (SDNN) and the vasovagal tonus index (VVTI).

Results No R-R intervals differences were found in the animals before the preanesthetic protocols were tested. The overall mean of 20 consecutive R-R intervals found APM was statistically higher than BPM (759.78 ± 232.41 ms and 628.29 ± 180.87 ms, respectively) (*P<0.05*). As unexpected result the AMG expressed significantly lower APM values than APG (718.54 ± 235.19 ms and 801.03 ±197.79 ms, respectively). The global MRR, SDNN, rMSSD and VVTI showed an increase values at APM (759.79 ± 109.20 ms, 190.86 ± 75.46 ms, 1080.0 ± 436.20 ms and 10.36 ± 0.95 respectively) in comparison to BPM (628.29 ± 92.44 ms, 132.43 ± 623.09 ms, 869.12 ± 438.43 ms and 9.69 ± 1.76) in both groups (AMG and APG).

Conclusions Acepromazine + pethidine and acepromazine + morphine used as preanesthetic medications possibly exacerbate the OCR in dogs elicited by digital compression. Even pethidine which was expected to have an anticholinergic effect, when associated to acepromazine demonstrated a significantly higher effect in slowing the heart rate than morphine. Thus, the capacity of a given anesthetic to increase the OCR effect should be considered before the anesthetic protocol is chosen for ophthalmic surgeries or

clinical manipulation of the globe, according to the cardiovascular condition of each patient in order to minimize the anesthetic risk.

Key words: autonomic nervous system, heart rate variability, vasovagal tonus index, ocular compression, preanesthetic medication, vagus nerve

1.1 INTRODUCTION

The oculocardiac reflex (OCR) is a neurophysiologic phenomenon in response to mechanical compression of the eyeball or traction applied to any of orbital and periorbital contents. In human beings a sudden reduction of more than 10 beats per minute[1] (10 to 20% compared with basal value)[2,3] is attributed to OCR activity when other causes are not observed. Until the moment, there is no set parameter for heart rate reduction that characterizes OCR in dogs.

OCR is accepted as a peripheral subtype of the trigeminocardiac reflex, since the afferent pathway basically involves the ophthalmic branch of the fifth cranial nerve (trigeminal nerve).[4] The efferent limb of the pathway is carried out by the vagus nerve, whose stimulation leads to a cardio-inhibitory[5] effect with negative chronotropic and inotropic responses.[1,3] In human beings the OCR was described triggering bradycardia, [1,2,4,5,6] atrioventricular block, [3] ventricular ectopy,[1,6] ventricular fibrillation[3,6] and even cardiac arrest.[1,3,6]

Heart rate variability (HRV) is an indirect technique to report and assess frequency and autonomic activity in heart.[7] This non-invasive tool measures intervals of time between every normal sinus beat (represented as R-R peak waves) on the surface of the electrocardiogram.[8] The analyses of HRV over very short periods (< 60s) is clinically useful to evaluate responses of cardiovascular system to autonomic variation.[9] In time-domain category at short-term of HRV field, the vasovagal tonus index (VVTI) leads information about high-frequency variations in heart rate (resulted from fast vagal influences).[10] Beyond this HRV analyses, there are others useful indexes calculated from segments that can reports cardiac system: mean of R-R intervals (MRR)[8], standard deviation of all normal-to-normal intervals (SDNN)[11] and the root mean square of successive R-R intervals differences (rMSSD)[11]. All these variables except VVTI are expressed in milliseconds. SDNN is often used to describe tonic parasympathetic outflow, while rMSSD indicates parasympathetic activity.[12]

The association of acepromazine and an opioid, mainly morphine or pethidine, is a common combination used in dogs.[13] Acepromazine is a phenothiazine tranquilizer that acts on the central nervous system and can improve the effect of other drugs.[14] Morphine is an opioid used to treat severe pain and as preanesthetic agent.[14] Pethidine is also an opioid, but with one-eighth the analgesic potency of morphine.[15] In spite of its analgesic properties, pethidine was first synthesized in 1939 as an anticholinergic agent.[16]

Few studies have been conducted to characterize the OCR in dogs.[1,17,18,19,20,21,22] the most recent investigated conscious patients.[22] However, to the authors knowledge, there is no controlled studies about the effects of drugs used in premedication on the OCR in dogs. As morphine and acepromazine can increase parasympathetic tonus causing bradyarrhythmias, and pethidine has anticholinergic effects, we predicted that the OCR might be enhanced by the anesthetic premedication with acepromazine and morphine, and minimized when acepromazine is associated with pethidine. There is no literature evoking OCR with association of different drugs using HRV analyses in dogs.

OCR can manifest serious consequences. [23] Any facial surgery especially Ophthalmic can evoke OCR. The purpose of this study was to determine the effects of the premedication with acepromazine associated to morphine or pethidine on the intensity of the OCR experimentally induced in dogs, using for assessment HRV analyses.

1.2 MATERIALS AND METHODS

1.2.1 Animals

Fourteen Beagle dogs (seven females and seven males) clinically healthy with the same age (seven years) were used in this study. The animals were from a small animal nutrition laboratory colony (LENUCAN- *Laboratório de Estudos em Nutrição Canina*) located at the Federal University of Paraná, Brazil. All dogs were born in the colony, underwent complete physical examination and complete blood count analysis including biochemical tests (urea, creatinine, alanine amino transferase, alkaline phosphatase and cholesterol). None of the dogs studied showed abnormalities in any of the abovementioned laboratory parameters. All of them were classified as I by the system of Physical Status from the American Society of Anesthesiologists (ASA).The procedures performed in this investigation were approved by the ethics committee of the Federal University of Paraná (Curitiba, Paraná state, Brazil).

1.2.2 Groups and evaluation times

Dogs were randomly assigned to one of two groups (n = 7 each). Group 1 (AMG) was premedicated with morphine (0.3 mg/kg) – Dimorf®(Cristália Produtos Químicos e Farmacêuticos, Itapira – São Paulo state, Brazil) and acepromazine (0.03 mg/kg) – Acepran® (Vetnil Indústria e Comércio de Produtos Veterinários, Louveira , São Paulo state, Brazil). Animals allocated in Group 2 (APG) were premedicated with acepromazine

(0.03 mg/kg) and pethidine (4 mg/kg) – Cloridrato de Petidina®(União Química Farmacêutica Nacional SA, São Paulo – SP, Brazil). The drugs in both groups were administered by intramuscular injection.

1.2.3 OCR stimulation

The ocular compression maneuvers followed a previously published protocol.[22] In brief; the compression maneuver itself has seven different stages, each lasting one minute (Table 1.1 and Fig 1.1). The protocol was repeated at two different moments: 1) Two hours before the administration of preanesthetic medication (BPM); 2) Fifteen minutes after the preanesthetic medication (APM).

Ocular compression was applied only in three stages (second, fourth and sixth), and between them, in the other four stages (first, third, fifth and seventh) dogs underwent only a gentle physical restraint. These four no-compression stages were like "rest intervals" between the ocular globe compression stages. The right eye (OD) was compressed first, followed by the left eye (OS) and finally both eyes (OU) together. The compression was performed by manual digital force pressing the thumb over the superior eyelid (Figure 1.1) by the same investigator (Vieira, TCS). No instruments were used to measure the strength applied, however the force was enough to cause a slight retrobulbar displacement (retropulsion) of the globes.

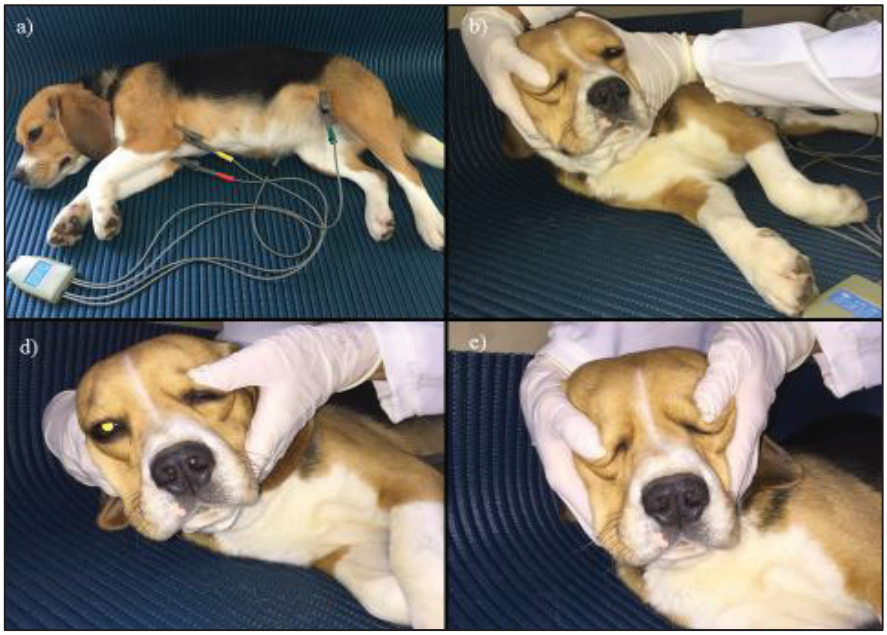


Figure 1.1. Different stages of the oculocardiac reflex investigation demonstrated in a representative Beagle dog. (a) One minute interval between each compression, only with physical restraint (Stages 1, 3, 5 and 7). (b) Right eye compression with

the thumb over superior right eyelid (Stage 6). (c) Left eye compression (Stage 4). (d) Compression of both eyes simultaneously (Stage 6).

Table 1.1. Protocol of eye compression maneuvers and pauses, repeated from previous study,[28] with Beagle dogs during two moments (before and after preanesthetic medications).

Stage	Time interval (min)	Procedure
1	0 -1	Gentle physical restraint of animal, no ocular compression
2	1 - 2	Compression on the right eye
3	2 - 3	Pause, with only gentle physical restraint of the animal
4	3 - 4	Compression on the left eye
5	4 - 5	Pause, with only gentle physical restraint of the animal
6	5 - 6	Compression of both eyes
7	6 - 7	Gentle physical restraint of animal, no ocular compression

1.2.4 Electrocardiogram analysis

Electrocardiograms were recorded using ECGPC, TEB (Brazilian Electronic Technology Ltda, São Paulo, SP, Brazil) among seven stages. Dogs were positioned in right lateral recumbency and the electrodes were attached to the skin on each of the four limbs with atraumatic crocodile clips (Figure 1.1). Measurements were derived from the lead II recording and only continuous runs of sinus rhythm traces were used. For the calculation of HRV and for all descriptive and inferential statistics, twenty consecutive normal complexes were selected, where the R-R intervals were measured after 40 seconds (in pilot study heart rate started to decrease from this moment) in each stage.

HRV was analyzed in the time domain and expressed as: 1) MRR, 2) SDNN, 3) rMSSD and 3) VVTI (VVTI = NL (sdRR)², which NL = natural logarithm of the variance and sdRR = squared standard deviation of 20R-R intervals). Values of MRR, SDNN and VVTI were expressed as means from all animals, at each stage, in both groups (APG and AMG) and moments (BPM and APM).

1.2.5 Statistical analyses

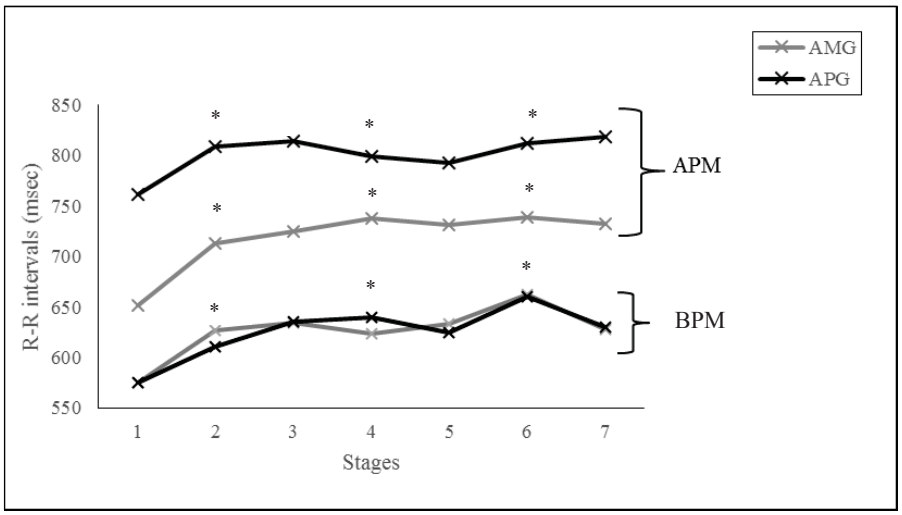
Statistical analyses were performed using the software Sigma XL version 7.0 (Kitchener, Canada). After ensuring the data were normally distributed (by Shapiro-Wilk test), paired t-test was used in each group (AMG and APG) to compare the effect of the OCR before and after drugs administration (BPM *vs* APM). A two-factor repeated measures ANOVA test was used to compare differences among stages in each group and

moment. To compare groups without differentiate stages (APG and AMG) in each moment (before and after-BPM, APM- drugs administration) unpaired t test was used. For HRV parameters (MRR, SDNN, RMSSD and VVTI); data were tested for normality using the Shapiro-Wilk test. These parameters did not show a normal distribution. Then, a Wilcoxon test was used to compare moments (BPM *vs* APM-before and after the drug protocols) in each drug group and a Mann-Whitney test to compare the drug groups (APG x AMG). All *P*-values are two-sided and were considered significant at $P < 0.05$. Values were expressed in milliseconds, except VVTI (which does not have any unit type).

1.3 RESULTS

1.3.1 Overall comparison of data

The following means were observed in general values of moments (BPM and APM) and groups (AMG and APG). To ensure that there was no difference in vasovagal tonus between groups in BPM, they were compared using unpaired t-test (*P = 0.59*). The mean of intervals between R-R waves increase significantly after preanestethic medication during the seven stages, (BPM 628.29 ± 180.87 ms and APM 759.78 ± 232.41 ms, $P < 0.0001$)(Graphic 1.1). APG expressed significantly higher R-R intervals (801.03 ms ± 197.79 ms) than AMG (718.54 ± 235.19 ms) at APM ($P < 0.0001$) (Graphic 1.1). Subsequence the first globe stimuli, in both moments and groups all stages had large R-R intervals (Graphic 1.1).



Graphic 1.1. Mean of 20 R-R intervals in milliseconds of each moment (BPM and APM) and group (AMG and APG) among the seven stages. Black lines represent APG (acepromazine + pethidine group) and grey lines represent AMG (azepromazine + morphine group). Observe in BPM (before preanesthetic medication), represented by circle markers, lower values. When anesthetic

premedication is administered (after preanesthetic medication - APM) R-R intervals increase and after the first eye compression (Stage 2), intervals became even more distant. Stages 2, 4 and 6 (marked with *) represent stages with ocular compression.

1.3.2 Stages in BPM

The following means were observed in BPM without eye compression (Table 1.2): Stage 1 (574.90 ± 147. 92 ms); Stage 3 (634.41± 178.30 ms); Stage 5 (628.79 ± 180.39 ms) and Stage 7 (629.08 ± 188.73 ms). Stage 1 was significant lower than Stage 3, Stage 5 and Stage 7 ($P = 0.00002$, $P = 0.000012$ and $P = 0.000017$ respectively). On the other hand, the other no compression stages had no differences between them: Stage 3 vs. Stage 5 ($P = 0.7107$), Stage 3 vs. Stage 7 ($P = 0.7312$) and Stage 5 vs. Stage 7 ($P = 0.9850$) (Table 1.3).

Compression stages (Stage 2 - OD, Stage 4 - OS and Stage 6 - OU) at BPM showed R-R intervals significantly higher than baseline ($P < 0.0001$). The following means were found: Stage 2 (618.64 ± 180.39 ms), Stage 4 (631.90 ± 171.02 ms) and Stage 6 (661.08 ± 195.70 ms) (see Table 1.2). Both eyes compressed had significantly larger R-R intervals than the first eye compressed (Stage 6 vs. Stage 2, at $P = 0.0056$) (Table 1.3). However, no differences were found between OU and OS compressed (Stage 6 and Stage 4, $P = 0.0608$ or isolated eyes (Stage 2: 618.64 ± 180.39 ms *vs.* Stage 4: 631.90 ± 171.02 ms) ($P = 0.3504$). The compression of isolated eyes did not result in statistically difference of stages without stimulus (Stage 3, Stage 5 and Stage 7) (Table 1.3). Stage 6 had significantly high R-R intervals than stages before (Stage 5) and after (Stage 7) without any ocular compression (Stage 6 *vs.* Stage 5 at $P = 0.0428$ and Stage 6 *vs.* Stage 7, $P = 0.0494$).

Table 1.2. Means and standard deviations from different group of drugs (AMG and APG) through seven stages in BPM in milliseconds. Stage 1: baseline, Stage 2: right eye compression, Stage 3: rest interval with only physical restraint, Stage 4: left eye compression, Stage 5: rest interval, Stage 6: both eyes compressed and Stage 7: final rest.

Group in BPM	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
AMG	574.90 ± 147.92	626.42 ± 174.26	633.93 ± 188.26	623.86 ± 175.90	633.30 ± 190.39	662.22 ± 212.17	628.00 ± 194.65
APG	574.9 ± 147.92	610.85 ± 154.55	634.90 ± 168.02	639.93 ± 166.23	624.27 ± 172.38	659.93 ± 178.46	630.17 ± 183.31
Total	574.9 ± 147.92	618.64 ± 180.39	634.41 ±178.30	631.9 ± 171.02	628.79 ± 180.39	661.07 ± 195.70	629.08 ± 188.73

AMG, acepromazine + morphine group; APG, acepromazine + pethidine group; APM, after preanesthetic medication.

Table 1.3. General comparison performed between different stages and respective P-values, without consider drug groups in BPM. Significate differences were marked (*).

Comparison	P-value
Stage 1 *vs* Stage 2	< 0.0001*
Stage 1 *vs* Stage 3	0.00002*
Stage 1 *vs* Stage 4	< 0.0001*
Stage 1 *vs* Stage 5	0.000012
Stage 1 *vs* Stage 6	< 0.0001*
Stage 1 *vs* Stage 7	0.000017*
Stage 2 *vs* Stage 3	0.2771
Stage 2 *vs* Stage 4	0.3504
Stage 2 *vs* Stage 5	0.4870
Stage 2 *vs* Stage 6	0.0056 *
Stage 2 *vs* Stage 7	0.4856
Stage 3 *vs* Stage 4	0.8646
Stage 3 *vs* Stage 5	0.7107
Stage 3 *vs* Stage 6	0.0925
Stage 3 *vs* Stage 7	0.7312
Stage 4 *vs* Stage 5	0.8343
Stage 4 *vs* Stage 6	0.0608
Stage 4 *vs* Stage 7	0.8533
Stage 5 *vs* Stage 6	0.0428*
Stage 5 *vs* Stage 7	0.9850
Stage 6 *vs* Stage 7	0.0494*

1.3.3 Stages in APM

Means after preanesthetic medication (APM) per stage is represented in Table 1.4. Only Stage 1 was statistically different from other stages (Table 1.5). No difference was found between stages with no ocular compression (Stage 3 *vs.* Stage 5, $P = 0.7108$; Stage 3 *vs.* Stage 7, $P = 0.7522$: and Stage 5 *vs.* Stage 7, $P = 0.4807$).

The compression of eyeball (Stage 6) showed an increase significantly of R-R intervals from basal values (Stage 1) ($P= 0.004$). Otherwise, in comparison of the stages with no stimulus it had no significant differences: Stage 2 *vs.* Stage 3 ($P = 0.68$), Stage 2 *vs.* Stage 5 ($P = 0.96$), Stage 2 *vs.* Stage 7 ($P = 0.46$), Stage 4 *vs.* Stage 3 ($P = 0.99$), Stage 4 *vs.* Stage 5 ($P = 0.72$), Stage 4 *vs.* Stage 7 ($P = 0.74$), Stage 6 *vs.* Stage 3 ($P = 0.75$), Stage 6 *vs.* Stage 5 ($P = 0.49$), Stage 6 *vs.* Stage 7 ($P = 0.99$). No significant differences were found in compression groups: OD *vs.* OS ($P = 0.69$), OD vs. OU ($P = 0.48$) and OS vs. OU ($P = 0.75$).

When the values of the first eye compressed are compared from each drug group (Stage 2 of AMG and Stage 2 of APG), Pethidine group (APG) had the highest R-R intervals (Pethidine is more reflexogenic than morphine in this study)

Table 1.4. Means and standard deviations from different group of drugs (AMG and APG) through seven stages in APM in milliseconds.

Groups in APM	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
AMG	651.81 ± 157.04	713.02 ± 200.27	724.43 ± 214.15	738.19 ± 209.58	730.97 ± 197.37	738.62 ± 212.28	732.76 ± 186.99
APG	761.86 ± 227.71	809.03 ± 265.07	813.79 ± 252.06	799.54 ± 267.26	792.84 ± 240.74	812.2 ± 272.91	817.91 ± 257.43
Total	706.83 ± 202.88	761.02 ± 239.37	769.11 ± 237.71	768.86 ± 241.69	761.90 ± 221.88	775.41 ± 246.81	775.33 ± 228.59

AMG, acepromazine + morphine group; APG, acepromazine + pethidine group; APM, after preanesthetic medication.

Table 1.5. General comparison performed between different stages and respective P-values, without consider drug groups in APM. Significate differences were marked (*).

Comparison			P-value
Stage 1	*vs*	Stage 2	0.0040*
Stage 1	*vs*	Stage 3	0.0009*
Stage 1	*vs*	Stage 4	0.0010*
Stage 1	*vs*	Stage 5	0.0022*
Stage 1	*vs*	Stage 6	0.0003*
Stage 1	*vs*	Stage 7	0.0001*
Stage 2	*vs*	Stage 3	0.6885
Stage 2	*vs*	Stage 4	0.6999
Stage 2	*vs*	Stage 5	0.9640
Stage 2	*vs*	Stage 6	0.4841
Stage 2	*vs*	Stage 7	0.4696
Stage 3	*vs*	Stage 4	0.9902
Stage 3	*vs*	Stage 5	0.7108
Stage 3	*vs*	Stage 6	0.7584
Stage 3	*vs*	Stage 7	0.7522
Stage 4	*vs*	Stage 5	0.7227
Stage 4	*vs*	Stage 6	0.7512
Stage 4	*vs*	Stage 7	0.7449
Stage 5	*vs*	Stage 6	0.4961
Stage 5	*vs*	Stage 7	0.4807
Stage 6	*vs*	Stage 7	0.9970

1.3.4 Heart rate variability

Median of HRV indexes of all stages in general per moment and group are represented in Table 1.6 and Table 1.7. All heart rate variability indices had the same behavior over the comparisons (Table 1.8). Values increased significantly after drugs administration in all stages (Graphic 1.2). No differences were observed between groups

(AMG *vs* APG) before drugs administration (Table 1.6). At APM, values from APG were significantly high when compared with AMG (Graphic 1.3).

Table 1.6. Median of all time domain HRV used in general and for each group with all stages in BPM expressed in milliseconds, except VVTI.

Variable	Time	General	AMG	APG
VVTI	BPM	10.29	10.27	10.32
SDNN	BPM	154.13	160.13	145.71
rMSSD	BPM	952.25	981.37	878.23
MRR	BPM	614.10	645.75	599.15

AMG, acepromazine + morphine group; APG, acepromazine + pethidine group; APM, after preanesthetic medication; BPM, before preanesthetic medication; MRR, mean of R-R intervals; rMSSD, root mean square of successive R-R intervals; SDNN, standard deviation of normal to normal intervals; VVTI, vasovagal tonus index.

Table 1.7. Median of all time domain HRV used in general and for each group with all stages in APM expressed in milliseconds, except VVTI.

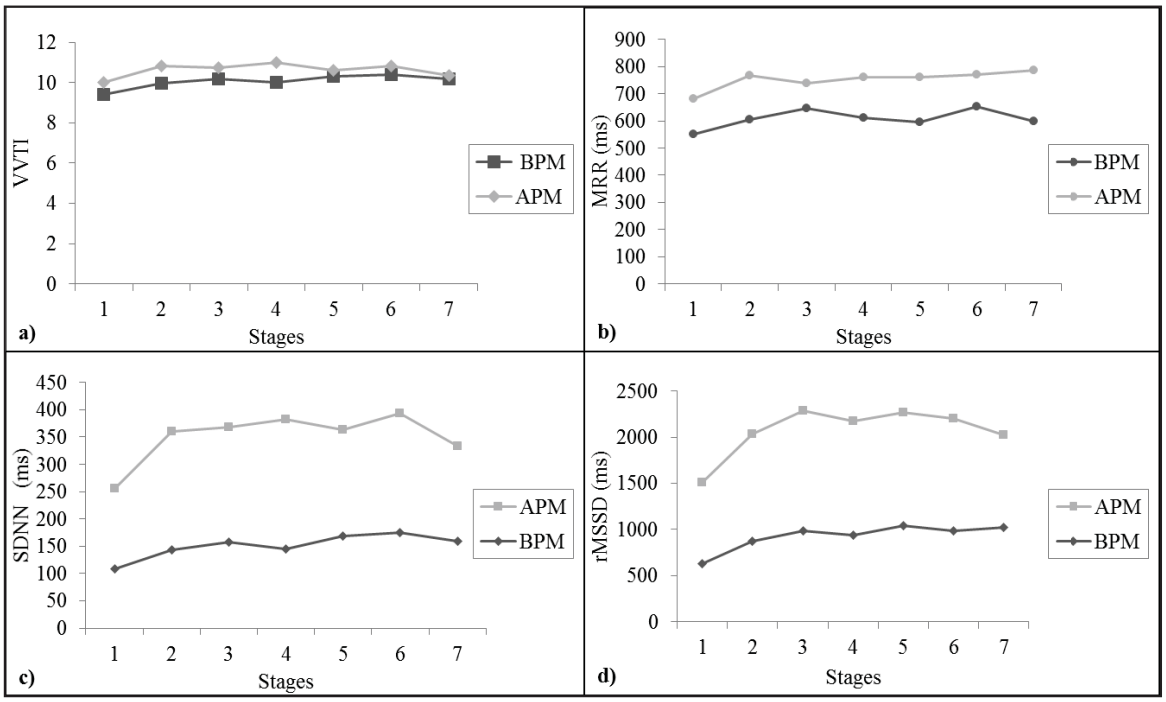
Variable	Time	General	AMG	APG
VVTI	APM	10.57	10.28	10.99
SDNN	APM	192.27	166.67	237.35
rMSSD	APM	1160.19	953.85	1291.74
MRR	APM	756.68	720.30	787.10

AMG, acepromazine + morphine group; APG, acepromazine + pethidine group; APM, after preanesthetic medication; BPM, before preanesthetic medication; MRR, mean of R-R intervals; rMSSD, root mean square of successive R-R intervals; SDNN, standard deviation of normal to normal intervals; VVTI, vasovagal tonus index.

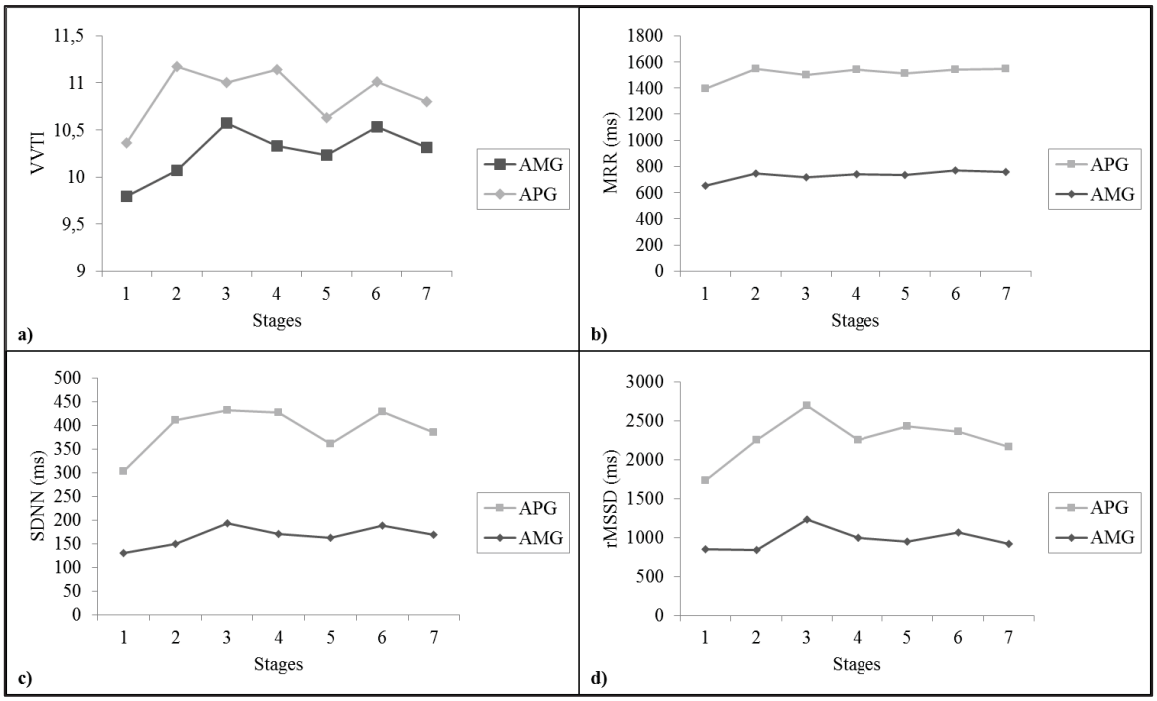
Table 1.8. Comparison between moments and drug groups with its respective P-values. Significances are signalized (*).

Variable	Comparison			P-value
VVTI	BPM	*vs*	APM	< 0.0001*
VVTI	AMG (BPM)	*vs*	APG (BPM)	0.0804
VVTI	AMG (APM)	*vs*	APG (APM)	0.0024*
SDNN	BPM	*vs*	APM	< 0.0001*
SDNN	AMG (BPM)	*vs*	APG (BPM)	0.0817
SDNN	AMG (APM)	*vs*	APG (APM)	0.0021*
rMSSD	BPM	*vs*	APM	< 0.0001*
rMSSD	AMG (BPM)	*vs*	APG (BPM)	0.0664
rMSSD	AMG (APM)	*vs*	APG (APM)	0.0005*
MRR	BPM	*vs*	APM	< 0.0001*
MRR	AMG (BPM)	*vs*	APG (BPM)	0.8036
MRR	AMG (APM)	*vs*	APG (APM)	0.0004*

AMG, acepromazine + morphine group; APG, acepromazine + pethidine group; APM, after preanesthetic medication; BPM, before preanesthetic medication; MRR, mean of R-R intervals; rMSSD, root mean square of successive R-R intervals; SDNN, standard deviation of normal to normal intervals; VVTI, vasovagal tonus index.



Graphic 1.2. Heart rate variability among the seven stages and moments (BPM- before preanesthetic medication, and APM- After preanesthetic medication): a) - Vasovagal tonus index (VVTI), b) MRR - mean of R-R intervals in milliseconds, c) SDNN – standard deviation of normal-to-normal intervals in milliseconds and d) rMSSD – root mean square of successive R-R intervals differences in milliseconds.



Graphic 1.3. Heart rate variability among the seven stages after preanesthetic medication (APM) of each group (AMG, acepromazine + morphine group; APG, acepromazine + pethidine group): a) - Vasovagal tonus index (VVTI), b) MRR - mean of R-R intervals in milliseconds, c) SDNN – standard deviation of normal-to-normal intervals in milliseconds and d) rMSSD – root mean square of successive R-R intervals differences in milliseconds.

1.4 DISCUSSION

The decrease in heart beat following opioid administration was already described as vagally-mediated.[14] In this study we found exactly this phenomenon; the R-R intervals in milliseconds after drugs administration in all stages increased significantly (Graphic 1.2). OCR was present in both moments (BPM); subsequent the first stimulus applied (Stage 2 in BPM and Stage 2 in APM) values arise from baseline (Stage 1) significantly (Graphic 1.1).

At BPM the compression of both eyes, OU (Stage 6) showed larger R-R intervals in milliseconds than compression of only OD (Stage 2). Some papers already show the augment of OCR when binocular compression was applied.[19,22] However, after the preanesthetic medication (APM) the compression of both eyes (Stage 6) was not significantly different from compression of isolated eyes (Stage 2 and Stage 4). Drugs effect might interfered the answering of OCR in Stage 2 and successive stages.

Stages in APM was not different between them (Table 1.2), except when compared with baseline (Stage 1). The OCR in APM was modified by drugs. We should also consider the age of the animals tested, they are considered elderly for its breed (7 years), which can interfere in vagus nerve effectiveness response.[11] OCR was described to decrease with progression of the age.[3] The vagus nerve might also went into fatigue, reducing the bradycardia observed and differences between stages in APM . Fatigue of vagus nerve was described at intervals of rest as long as 76 seconds.[24] Fatigue and vagal escape are physiologic and compensatory mechanism against excessive vagal stimulations and depends on the period of time that vagus nerve stays refractory to repetitive stimulation.[25] Generally fatigue reduce the intensity of bradycardia and probably the OCR effect. Vagal escape can reach plateau phase in 7 to 25 seconds, when bradycardia falls spontaneously even with constant force.[25]

Another possibility was the concept of co-activation of sympathetic and parasympathetic system in OCR.[26] Sympathetic component may exist in OCR in a compensatory form after the stimulation of the parasympathetic. The protocol time between every stage of one minute was establish by previous studies,[22] since OCR can last among 20 and 60 seconds.[28]

Anticholinergic drugs are usually effective in preventing bradycardia during surgery.[27] Pethidine was first synthetized to have anticholinergic properties.[16] In human beings pethidine was described as the only opioid that can block the activity of the vagus

nerve.[29] Despite of its controversial origin and chemical structure, pethidine appears to have the most cardiac depressant of the opioids in dogs even in low doses (2.5 mg/kg).[30] The clinical significance heart effects of pethidine is not clearly characterized. Some authors observed cardio-inhibition[30] others affirm tachycardia[31,32] a few others no change at all.[33] One previous research[34] observed in dogs that pethidine in low doses has only mild effects (2mg/kg) without changes in heart rate, while larger doses (6 mg/kg) cause an increased heart rate until 15 minutes of administration.

Among the opioid drugs commonly used in veterinary medicine, it is known that the administration of morphine depresses cardiovascular function in many species.[35] In our study, AMG hoped to trigger a large reduction in R-R intervals and increase the OCR effect more than APG, but the opposing effect occurred. Our research used clinical drug doses considering the references values of intramuscular administration (Pedidine: 1- 10 mg/kg; Morphine 0.1-1mg/Kg),[32] the reason was to do not exacerbate the sedation and observe OCR effects properly.

Time domain variables of HRV (SDNN, VVTI, rMSSD) report the activity of cardiac system. A raise in vagal or parasympathetic tonus reduce heart rate,[8] and it is followed by increase of HRV values.[36] HRV can possibly be a marker of homeostasis lost. When reduced is a strong indicator of healthy adverse events.[7] SDNN represents sympathetic and parasympathetic activity, not so specific for vagal tone, because did not permit distinguish how of this changes are from an increase in sympathetic tonus or outflow of vagal stimulus.[12] Observe that in APM group, SDNN and rMSSD augmented R-R intervals in milliseconds (Table 1.7 and Graphic 1.2). The same is observed between groups in APM, pethidine demonstrate more pronounced OCR (see Tables 1.7 and Graphic 1.3). AMG induced less bradycardia than APG, effect observed also in HRV. Studies developed previously[37] proposed reference values for short-term (60-min) values of HRV at 95% confidence: 180 to 238 ms for SDNN, and 214 to 305 ms. This research found age related increase in SDNN values and decrease of rMSSD while dogs get older. It is important to enforce that research did not use a uniform population, they used a different breeds, ages and weight of dogs. We found values of SDNN inside this proposed parameters, but rMSSD does not matches, ours values were very high, possibly for OCR and drugs effect. Considering the standard deviation found for rMSSD, our sample result in varying values but all tends to increase among stages.

High HRV indicates adaptation and an efficient autonomic mechanism, while lower is a signal of insufficient adaptation of the autonomic system.[7] Furthermore, high

HRV values indicate change in sympathetic and parasympathetic balance toward increased vagal tonus.[7] Values found for VVTI after the preanesthetic medication shows that parasympathetic system was raised by drugs especially in APG group. VVTI vary with available research, does not seem to be consensus about references value in normal dogs. Previous studies were performed assessing VVTI, but most of them in cardiac and heart failures patients dogs.[11] In healthy dogs the VVTI, with values close to 8, do not have any significance effect on behavior response during clinical examination.[38] Dogs with dilated cardiomyopathy had for VVTI a mean of 5.2. varying between 5.1 and 7.7 depending on the ISACHC class (International Small Animal Cardiac Health Council classification).[11] Dogs with congestive heart failure exhibit values less than 7 and dogs with mitral insufficiency had lower than 6.82.[11] Previous study performed with healthy dogs evaluate the effects of propofol and sulfentanil on cardiac autonomic system.[39] VVTI similar was found to this experiment: values close to 9, but dogs went through preanesthetic medication and intravenous drug. Doxey and Boswood (2004),[11] found brachycephalic breed with VVTI more than 8.5, while non - brachycephalic dogs was less than 8.1. In this case, we use mesaticephalic skull, did not agreeing with parameters found by Doxey and Boswood (2004). In our experiment VVTI augment with the administration of drugs, and additional the large R-R intervals, APG showed higher values.

RR intervals in ECG surface are inversely proportional to heart rate. Further is the interval lower will be the heart rate. Converting the values to heart frequency without differentiate the drug, we found decrease of 20% in BPM to APM; 34,8 % in dogs with binocular compression and preanesthetic drugs from baseline (Stage 1 in BPM to Stage 6 in APM) and 17% of reduction in stages with binocular compression (Stage 6 from APM and Stage 6 from BPM). Previous studies found a reduction of 26% [22] in conscious dogs and 20% in a dog with a orbital expansion of a choroidal melanoma.[20] OCR induced by zygomatic arch fracture in dog was also described with atropine tests as restore of normal heart rate.[21]

The type of stimulus could influence the intensity of OCR. One experiment found the type of stimuli to evoke OCR, using micro displacement myograph transducer. This equipment was sutured and connected in extraocular muscles in anesthetized children undergoing strabismus surgery. Acute traction equal or more than 150 g sustained for a minimum of 20 seconds followed by acute release demonstrated to be significantly more reflexogenic than the slowly and progressive stimulus.[25] Compression of both eyes are more profound than monocular in dogs.[19,22]

Even with many maneuvers to abolish OCR, none of them is safe and reliable. There is still an absence of a gold-standard method for OCR prevention.[40] Bradycardia from OCR effects in dogs is reduced by central by inflation of the lungs.[18] It would be used in ophthalmic procedures to increase the frequency and reduce OCR.

Arrhythmia, prolongation of the interval P-R, negative deflections of the p-wave, ventricular escapes, extrasystoles, asystoles and other rhythm disturbances were not observed in this study. Heart rate alterations were the only change observed.

A limitation of the present study was the absence of assessing different acepromazine doses and a sedation degree score. The purpose was simply to determine the possibility of the anesthetic drugs to exacerbate the occurrence of a physiological OCR. The same dose of acepromazine was present in both groups (AMG and APG) and different effects were observed. Pharmacological agents as narcotics was already described to predispose OCR by the inhibition of the synaptic in nervous system.[14] Despite that pethidine has an atropine-like structure and some reports even described that it can produce tachycardia, in this study it showed the ability of inhibit sinus node activity and possibly potentiate the OCR more than morphine.

It is important search for anesthetic drugs and techniques that can reduce the incidence of OCR, that was described as difficult to control during ophthalmic surgeries with normal doses of atropine in dogs.[17]

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CAPÍTULO 2

The effect of eye stimulation using graded external compression force in the experimental oculocardiac reflex in beagle dogs

Resumo

O objetivo deste estudo foi investigar a existência de um potencial aprofundamento do ROC à medida que o estímulo de compressão ocular é aumentado progressivamente. Foram estudados oito cães da raça Beagle com aproximadamente um ano e dois meses de idade clinicamente sadios. O estímulo para o reflexo foi aplicado através do analgesímetro eletrônico de von Frey sobre a pálpebra superior do olho esquerdo (OS) dos cães. O equipamento teve uma adaptação na ponta para que não lesionasse ou causasse dor ocular ao animal. Foram aplicadas quatro forças diferentes: 1) 200 gramas, 2) 300 gramas, 3) 400 gramas e 4) 800 gramas. O experimento foi realizado em quatro dias diferentes, um para cada força testada nos oito animais. O eletrocardiograma monitorou todas as etapas do experimento. Primeiramente, foi realizado durante 1 minuto o registro do traçado cardíaco basal, onde o cão permaneceu em decúbito lateral direito somente com uma leve contenção. Em seguida, novamente com a duração de 1 minuto, o animal foi submetido a compressão ocular. A variabilidade da frequência cardíaca através do índice de tônus vasovagal foi usada para análise dos dados obtidos. Vinte intervalos entre cada batimento cardíaco (picos de onda R ou intervalo R-R) em milissegundos foram usados. Todas as forças de compressão foram suficientes para desencadear o reflexo. O ROC se demonstrou ser um evento força-dependente até um limiar de 300 gramas aplicado. A partir deste ponto a compressão ativa mecanismos compensatórios do reflexo, ou possivelmente desencadeia dor, resultando em taquicardia.

Abstract

Objective To determine if oculocardiac reflex (OCR) in conscious dogs progressively augments with increasing ocular compression force.

Animal studied Ten clinically healthy adult beagle dogs.

Procedures. An electronic electrocardiogram recorded the rhythm and heart rate without interruption during the procedure. External compression was applied in left eye by a modified electronic von Frey anesthesiometer with a modified probe. Four different pressures (200g, 300g, 400g and 800g) were applied in four different non-consecutive days (one-week intervals) in the same animals. The compression was performed using the device over the superior eyelid. First, the baseline heart rate values from each dog were recorded for one minute. Subsequently the electrocardiogram was recorded when compression of the eyeball was being applied during one minute. Heart rate variability was used to analyze the electrocardiogram tracings; twenty consecutive normal complexes of beat were used for each dog.

Results All pressures were sufficient to trigger OCR. The median R-R intervals found with a pressure of 300g was higher (645 ms, IQR: 223.5 ms) than others pressures (200g: 598.5 ms, IQR: 184 ms; 400g: 500 ms, IQR: 142 ms and 800g: 461.5 ms, IQR: 113 ms).

Discussion and Conclusions Any external eye compression with weight of 200 g or more was sufficient to elicit an OCR response in conscious dogs. A pressure of 300g was sufficient to trigger the peak of OCR without causing too much distress in dogs. Pressures of of 400g and 800g activated compensatory mechanisms to avoid OCR probably linked to an adrenergic response induced by the animal's discomfort during the test.

Key words: von Frey, autonomic nervous system, heart rate variability, ocular compression, vagus nerve

2.1 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).[1] The OCR can be life threatening and may occur in complex forms with arrhythmias, atrioventricular blocks, ventricular fibrillation, and even asystole.[2,3] Oculocardiac reflex is well documented in human patients with facial trauma and fractures,[4,5] orbital tumor,[8] subconjunctival injections,[9] and ocular surgeries especially in pediatric surgeries to correct strabismus.[2] In dogs, OCR was described in patients with choroidal melanoma extending to the orbit,[8] and zygomatic fracture.[9] Additionally, experimentally-induced-OCR was well-characterized in anesthetized[10, 11] and conscious dogs[12].

The effect of manual pressure on one or both eyes on the OCR in dogs was previously studied by our group.[12] However, in our previous investigation the force of manual compression was not measured. The electronic von Frey anesthesiometer was designed to perform quantitative sensory testing, measuring the pressing force applied on a given tissue.[14,15]

Heart rate variability (HRV) is a non-invasive technique to assess autonomic nervous system function 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 (in electrocardiogram is the interval between heart beats).[15] The VVTI provides information about variations in heart rate elicited from fast vagal activation, and was described in healthy, conscious dogs[16,17]

The purpose of this study was to investigate the correlation between the amount of pressure applied onto the canine eye and the OCR, utilizing the electronic von Frey anesthesiometer with a modified, non-traumatic, rubber probe. Our hypothesis was that OCR would be progressively increase with increasing external forces of compression

2.2 MATERIALS AND METHODS

2.2.1 Animals

Ten healthy one-year-old beagle dogs (five females and five males) from an animal nutrition laboratory canine colony (LENUCAN) located at the Federal University

of Paraná, Brazil were used in this study. All dogs had undergone a complete ophthalmic and general physical examinations. All animals were determined to be healthy and free of any ophthalmic or systemic disease. This study was approved by the ethics committee of the Federal University of Paraná (Curitiba, Paraná state, Brazil).

2.2.2 Procedures

The study was performed in four non-consecutive days. Dogs were positioned in a right lateral recumbency, and were connected routinely to an electrocardiograph (ECG) machine (ECGPC-TEB, Brazilian Electronic Technology Ltda., São Paulo, SP, Brazil). The electrodes used were alligator-type with no teeth to reduce the discomfort to the animals. These were fixed directly on the skin of the animals, making it necessary the use of alcohol to allow better conduction of electrical stimuli. The cranial electrodes were placed at the elbow joint (yellow electrode in the left forelimb and red electrode in the right forelimb), while the caudal electrodes were placed at the knee joint (green electrode in the left hind limb and black electrode in the right hind limb).

On the resulting electrocardiograms, the intervals from the peak of one QRS complex to the peak of the next (also known as R-R intervals) were measured in order to assess the heart rate (HR).

2.2.3 OCR Stimuli

Ocular compression was performed only on the left eye using an electronic von Frey anesthesiometer (EFF 301, Insight, São Paulo - Brazil). Originally, the equipment possess with interchangeable plastic rigid tips or flexible plastic filaments (or von Frey hairs) to perform sensory tests. We used a modified, 2.4 x 2.4 x 2.4 cm styrene-butadiene rubber probe, (Figs 2.1 and 2.2) weighing 25 g. The probe modification was intended to increase the size of the tip of the original probe in order to evenly distribute the force applied onto the globe and to make it more atraumatic to the eye. The weight of the new adapted rubber modified probe was electronically adjusted (tared) to zero. Mechanical compression was performed by applying the rubber probe to the skin over the superior eyelid. The eye was compressed with four different pressures: 200, 300, 400 and 800g. In each animal, first the baseline HR was recorded for the duration of one minute, followed by one minute of compression. The pressure forces were applied in four different non-consecutive days (in one-week intervals) in the same animals, in the following order 200, 300, 400 and 800g each day.

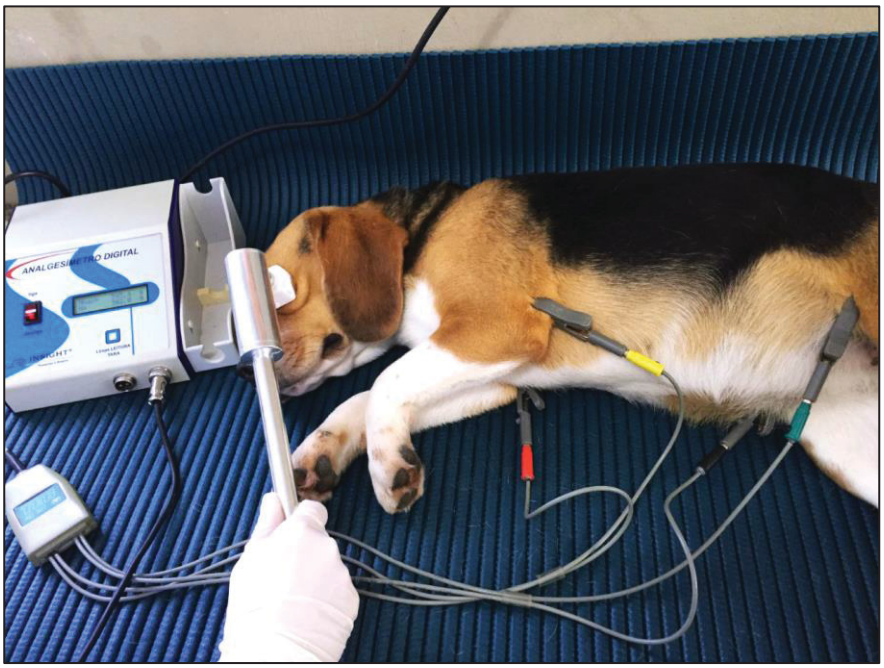


Figure 2.1. Compression on the left globe over the superior eyelid using a modified electronic von Frey anesthesiometer, while having an electrocardiogram being recorded during the procedure.

Figure 2.2. A close up view of the modified rubber probe of the electronic von Frey anesthesiometer device.

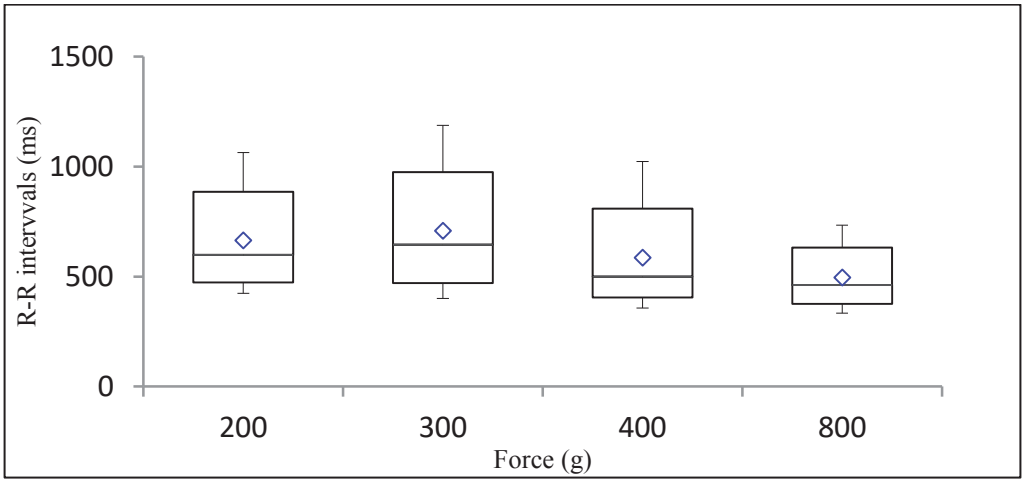
2.2.4 Statistical analyses

Twenty consecutive normal R-R intervals were measured on the electrocardiogram. The VVTI was calculated: VVTI= LN [VAR (R-R1- R-R20)], where LN is natural logarithm, and VAR is the variance. Data were tested for normality using the Shapiro-Wilk test. Since the data did not follow a normal distribution, a Kruskall-Wallis test was used to compare the R-R intervals obtained with all different ocular compressions. To

demonstrate whether or not an OCR was being present at each condition, a Wilcoxon test was used comparing each baseline R-R intervals with the ones acquired when different pressure forces were being applied in the same individuals. P-values were considered significant at P <0.05. Software Sigma XL version 7.0 (Kitchener, Canada) was used in all statistical analysis.

2.3 RESULTS

Overall, all dogs were very calm during the study and tolerated well all external ocular compression forces, with the exception of two dogs that showed little discomfort in response to the highest compression force. The R-R interval medians for each force of compression are listed in Table 2.1 and Figure 3. There was a statistically significant difference between all basal median values, obtained in response to all applied forces (P<0.0001). R-R intervals significantly increase with increasing force 200g: 598.5 ms, IQR: 184 ms (423 - 1063 ms) and 300g: 645 ms, IQR: 223.5 ms (400 – 1187 ms) (P<0.0001). Conversely, compression forces of 400g and 800g demonstrated lower median values, 400g: 500 ms, IQR: 142 ms (357 – 1023 ms) and 800g: 461.5 ms, IQR: 113 ms (333 – 733 ms). Median VVTIs of 200g and 300g, respectively: 9.08, IQR: 0.81 (8.5 – 10.7) and 300g: 9.90, IQR: 1.1 (9.13 – 10.85) were higher than when heavier forces were applied; 400g: 8.86, IQR: 0.5 (7.73 – 10.51) and 800g: 8.5, IQR: 0.9 (7.18 – 9.22) (P = 0.00027).



Graphic 2.1. Median of R-R intervals from each graded force in milliseconds. Note that R-R intervals increase with increasing forces of compression up to 300g. After this point increasing forces decrease the R-R intervals.

Table 2.1. Median and interquartile range (IQR) from different stimulus applied in left eye represented in milliseconds. Vasovagal tonus index (VVTI) values of all forces of the study. Baseline representing values of all animals without compression of the eyes.

Force(g)	Median (ms)	IQR	VVTI
None (baseline)	473	126	7.96
200	598.5	184	9.08
300	645	223.5	9.90
400	500	142	8.86
800	461.5	113	8.50

2.4 DISCUSSION

Previous investigations tried to determine the incidence of OCR in ophthalmic surgeries performed with the use of neuromuscular blocking agents NMBA.[13] Although the OCR effect is minimal in anesthetized dogs with MMBA, many ocular and adnexal surgeries are routinely perfomed without the use of NM block, hence the importance of this study.

The heart does not work as a continuous pump, each heartbeat is an event influenced alternatively by sympathic or parasympathic autonomic nervous systems.[18] HRV assessment is able to describe oscillations in heart rate resulted from fast vagal influences,[19] in the case of our investigation VVTI was used. Mooonamart *et al*. (2012),[16] and Doxey (2004)[17] found VVTI values from healthy dogs close to 8, similar to the baseline values found in our study.

The lower compression force used (200g), was already sufficient to elicit OCR in conscious dogs, likewise, a force of 300g was the most reflexogenic force applied in our study. Increasing VVTI values were observed until the 300g force was applied, after this point VVTI started to decrease with increasing weights. R-R interval values fell substantially when 400g and 800g were used. The possible explanation for these results would be the existence of a response limit, an intrinsic compensatory mechanism, in the autonomic nervous system for external compression of the globe, such as a sort of "autonomic co-activation system" that would be able to halt OCR. The possibility for the existence of such a system has been hypothesized elsewhere.[20] Another possible explanation would be that when higher external compression weights, i.e. 400 and 800g were used, the resulting ocular pain and discomfort activated the nociception system, with a subsequent adrenergic response causing the cardiac frequency changes.[21] If the animals

used in this experiment were anesthetized, the effects of graded stimuli probably would be different, since the pain threshold would probably be higher. Perhaps, if pain was not an issue, OCR would continuously increase with the higher forces of compression used in our investigation. On the other hand, with anesthesia, some of the drugs used could also affect the OCR, perhaps inhibiting or exacerbating it. For instance, inhalation anesthetic drugs might have different degrees of vagolytic action in dogs: Desflurane showed to be greatest followed by sevoflurane, isoflurane, enflurane and for least halothane.[22] In human beings, sevoflurane and desflurane are safe to use in strabismus surgery in children patients.[23] Opioids[24] in general and propofol,[25] widely used, potentially lead to bradycardia. Indeed, increasing doses of sufentanil proportionally decrease the heart rate in dogs.[26] Additionally, anticholinergic drugs as atropine and glycopyrrolate in dogs also reduce the risk of OCR occurrence.[13] In human beings ketamine showed inhibitory effect in parasympathetic receptors.[27] While in the medical field, there is an active search for a technique or anesthetic drug that can safely prevent OCR, there are fewer studies in veterinary medicine further investigating, this complication. Autonomic balance is not identical in dogs and human beings.[22]

The type of stimulus of mechanical compression of the eye influences the magnitude of OCR. Binocular compression deliver more profound OCR in comparison of monocular.[10,11] The electrostimulation of 5th cranial nerve dissected reveals similar reflex of monocular compression in dogs.[10] Acute traction of extraocular muscles is more reflexogenic than a smooth or gradual traction.[28] Graded stimuli progressively evoked the OCR reflex, using traction forces in extra-ocular muscles that varied from 50 to 250g in rabbits[29] and 150 to 300g in children[28]. We applied acute mechanical compression (and release) of 200g in one eyeball and found significantly augment in R-R intervals, a binocular compression might deliver even more effect of OCR.

Our investigation was the first to characterize canine OCR by graded stimuli with controlled ocular compression forces. We also showed the OCR is already elicited in monocular compressions of 200g in conscious dogs and its intensity is clearly force-dependent with compression forces up to 300g. One limitation of this study is the absence of blood pressure measurements since Mewly *et al.* (2015)[30] proposed that OCR might also have a hypotension component.

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3. CONSIDERAÇÕES FINAIS

O Reflexo oculocardíaco é um evento imprevisível sem incidência definida em cães com consequências possivelmente fatais. Esta pesquisa demonstrou que o reflexo atua com sinergismo em associação a algumas drogas anestésicas no cão. A força de compressão mecânica aplicada sobre o bulbo ocular é um fator determinante na gravidade do reflexo, sendo imprescindível essa informação aos cirurgiões na manipulação das estruturas que são inervadas pela raiz oftálmica do nervo trigêmeo. Assim como na Medicina, devemos buscar métodos "ouro" para minimizar os efeitos do ROC e evitar óbitos sem explicações durante procedimentos em face. O ROC pode ter efeito de taquicardia compensatório após estímulo excessivo ou escape vagal no cão. Este estudo é inovador em seu setor, visto que associou conceitos aplicados à cardiologia, estudo do balanço autonômico e fármacos anestésicos de rotina.

4. ANEXO

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 045/2015, referente ao projeto "Caracterização do reflexo oculocardiaco de cães submetidos à medicação pré-anestésica", sob a responsabilidade de **Thayane Cristine Santos Vieira** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau B de invasividade, em reunião de 17/06/2015

Vigência do projeto	Agosto de 2015 a agosto de 2016
Espécie/Linhagem	Cães de diversas raças
Número de animais	20
Peso/Idade	Variado
Sexo	Ambos
Origem	Animais atendidos no Hospital Veterinário da UFPR

CERTIFICATE

We certify that the protocol number 045/2015, regarding the project "**Characterization of the oculocardiac reflex in dogs submitted to pre-anesthesia medication",** under **Thayane Cristine Santos Vieira** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with B degree of invasiveness, in session of 06/17/2015

Duration of the project	August 2015 to August 2016
Specie/Line	Dogs of different breeds
Number of animals	20
Wheight/Age	Varied
Sex	Both
Origin	Animals from Veterinarian Hospital of UFPR

Curitiba, 17 de Junho de 2015.

Ananda Portella Félix
Presidente CEUA-SCA

Simone Tostes de Oliveira Stedile
Vice-Presidente CEUA-SCA

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