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Original article

Optimizing the experimental environment for dog telemetry studies

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Abstract

Introduction: The objective of this study was to test the influence of housing conditions on hemodynamics during cardiovascular general pharmacological studies. Our goal was to optimize both the quality of the data through an optimization of the physiological conditions, as well as to ensure the dog's well-being in general pharmacological studies. **Methods:** Two groups of four dogs were equipped with radiotelemetry transmitters and continuously monitored in two different housing models. Model I consisted of 4 cages, two on each site of a corridor. Model II consisted of 4 cages positioned in a row, where the bordering cages were not separated with a metal plate. The physiological status of the dogs in the different housing models was based on the frequency of vocalizations and the average resting heart rate, as well as video monitoring. **Results:** The housing arrangement during the study had a remarkable effect on the hemodynamics measured. The hemodynamic parameters were best when the dogs were housed with their usual run mate. In this setting, they have impressively low average heart rates of about 60 bpm during the entire study, was associated with fewer vocalizations. **Discussion:** This study demonstrated that the quality of the acquired cardiovascular data for conscious dogs is dependent on the pen configuration and group make-up during a study.

Keywords: Methods; Refinement; Conscious dog; Telemetry; Housing conditions

1. Introduction

The model preferred for cardiovascular studies according to the ICH S7a guideline is the conscious dog. The gold standard approach for studying conscious dogs is the use of a telemetry system to record cardiovascular parameters, i.e. blood pressure, left ventricular pressure, heart rate and ECG. The rationale for performing these studies in conscious dogs is based on the lack of possible influence of anesthesia on the parameters being measured. However, the conscious state is inherently responsive to environmental stimuli such that a higher spontaneous variability of the measured parameters must be taken into account. Thus, the quality of these types of cardiovascular studies is likely dependent on the degree of training of the animals used and the environment during a study. To show subtle drug-induced changes in heart rate, for example, it is essential to have a relatively constant heart rate without too many interruptions causing excitement (Guth, Germeyer, Kolb, & Markert et al., 2004).

Furthermore, a stable heart rate is desirable to facilitate correct QT detection and heart rate correction (Meyners & Markert, 2004). An unstable heart rate therefore detracts from the detection of a possible prolongation of QT interval.

The term refinement signifies the modification of any procedure, from the time a laboratory animal is born until its death, so as to minimize the pain and distress experienced by the animal and enhance its well-being. As mentioned by the members of the Joint Working Group on Refinement (Penny Hawkins et al., 2004), the ideal environment would allow for housing in stable, compatible groups.

Optimizing animal welfare is not only important from the viewpoint of ethics; it is also a prerequisite for conducting good quality studies. The experience of pain and other stress will result in physiological changes (e.g. heart rate) that increase the variability of the experimental results and a loss of sensitivity. A high variability of heart rate, for example, is often caused by excited or stressed dogs. Thus, it is in the interest of good science to ensure that conditions regarding animal housing are optimized. Important lessons have been learned about how to

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validate methods. The need to have predictive models available before validation is performed: the necessity to take the variability of the animal-based data which is to be used as the validation standard into account, and the need to have well-managed validation programs available (Purchase et al., 1998). Future progress will depend on the development of novel methods. The use of animals, especially dogs or monkeys, can only be justified, when clinically relevant data can be gained from few, but well-designed in vivo studies (Luft & Bode, 2002).

This can only be assured when the dogs feel comfortable and aren't stressed which also reflects the housing conditions. Enrichment activities can include group housing when possible, rather than individual cages and use of materials similar to those found in their natural habitats. Once the study is in progress, it is important that the laboratory staff is well trained and competent in the handling of the species that is being used.

One of the additional potential influences that may affect the hemodynamic status of the animals is the exact makeup of the group to be studied based on sex, family hierarchy and physical orientation to the other group members. It was the purpose of this study to assess the role of these factors on hemodynamic stability of the dogs, as an indicator of well-being and possible stress.

2. Methods

2.1. Animals

Treatment of the animals followed the German Law on the Protection of Animals and was performed with permission from the State Animal Welfare Committee.

Trained labrador dogs (male or female), at least 1.5 years of age, with body weights between 22 to 30 kg bred at Boehringer Ingelheim Pharma GmbH and Co. KG, Biberach were used. The dogs were group-housed as pairs of 2 in separate cages and had access to water ad libitum and were fed a standard dog diet once daily. They also had daily exercise periods of at least 1 h, each afternoon.

To monitor the health of the animals, we collect blood samples every 3 months to determine the blood count and clinical parameters, including electrolytes and kidney parameters.

The dogs were trained in the telemetry lab beginning 3 months before surgery, which was performed when the dogs were at least 1.5 years old.

2.2. Telemetry system

The telemetry system used to measure cardiovascular parameters is manufactured by Konigsberg Instruments, Inc. (Pasadena, CA) and marketed by RMISS (Deleware). It consists of 5 major components: a) an implantable unit; b) a receiver (antenna) located in the animal's cage together with an amplifier; c) ambient pressure monitor to measure atmospheric pressure; d) a PC-based "base station" to receive and process the amplified signals; e) a PC-based data acquisition system (NOTOCORD Hem 3.5) to process signals.

The implantable unit ("T27" total implant) consists of (1) two high fidelity pressure transducers (5.0 and 4.0 mm diameter), (2) an ECG cable, (3) micro-power battery-operated electronics that process and digitize the information from the pressure transducers and the ECG lead, (4) a radio-frequency transmitter that sends the signals to the telemetry receiver, and (5) a battery. A small cable projecting from the transmitter contains a magnetic switch that allows the device to be turned on and off. Prior to implantation the zero value of the two pressure transducers are calibrated using a manometer and 250 mm Hg is set to 5 V.

2.3. One-time surgical implantation

The transducers of the T27 implant were calibrated and the unit was sterilized using a low pressure ethylene oxide process prior to implantation.

Dogs were anaesthetised with a combination of Rompun (xylazine hydrochloride, 1 ml/10 kg, i.v.) and Ketavet (ketamine hydrochloride, 0.7 ml/10 kg, i.v.) after premedication with Atropine sulfate (0.04 mg/kg i.m.) and ventilated with 66% O_2 and 1%–1.5% isofluorane at a ventilation rate of 14/min. All procedures were performed under aseptic conditions using sterilized equipment.

The dogs were placed in a lateral recumbency with the left side facing the surgeon. An incision was made between the fifth and sixth intercostal space. A small pocket was opened in the abdominal wall for implantation of the transmitter, battery housing, and induction switch coil. The cables with both pressure transducers and ECG leads extending from the ventrally implanted transmitter were guided subcutaneously to the lateral incision. The antenna was guided subcutaneously from the transmitter location towards the spine and then runs parallel to the spine for ~25 cm. The initial ventral incisions required for battery and transmitter placement were closed. A left thoracotomy was performed between the fifth and sixth intercostal space to expose the left ventricle apex for insertion of the left ventricular Konigsberg transducer.

The aorta pressure transducer was implanted next. The aortic transducer, which also served as one electrode of the ECG, was inserted into the thoracic aorta just below the aortic arch. The transducer was sutured into place and blood flow was restored.

The lung was then inflated and the intercostal muscles were sutured closed and the pneumothorax evacuated. Chest incisions were closed.

The gas anaesthesia was then turned off and dogs were allowed to wake up. Analgesics and antibiotics (Temgesic and Duphamox® LA, Amoxicillin-Trihydrat) were administered for 2 days (Temgesic) and 10 days (Duphamox) following the procedure to support a good recovery and to ensure that the dog has no postoperative pain, a transdermal plaster (fentanyl, 25 μg/h) was placed on the skin for 2 days. Dogs are allowed to recover for at least 21 days before experiments using test substances are initiated.

2.4. Runs in the telemetry lab

Dogs used for studies in which the hemodynamic and ECG data are collected telemetrically are normally group-housed at