The Veterinary Journal 207 (2016) 140–146

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Surgical implantation and functional assessment of an invasive telemetric system to measure autonomic responses in domestic pigs

A. Krause [a,1], M. Zebunke [a,1], O. Bellmann [b], E. Mohr [c], J. Langbein [a,*], B. Puppe [a,d,*]

[a] Institute of Behavioural Physiology, Leibniz Institute for Farm Animal Biology (FBN), D-18196 Dummerstorf, Germany
[b] Scientific Organisation and Service, Leibniz Institute for Farm Animal Biology (FBN), D-18196 Dummerstorf, Germany
[c] Animal Health and Welfare, Faculty of Agricultural and Environmental Sciences, University of Rostock, D-18059 Rostock, Germany
[d] Behavioural Sciences, Faculty of Agricultural and Environmental Sciences, University of Rostock, D-18059 Rostock, Germany

ARTICLE INFO

Article history:
Accepted 24 October 2015

Keywords:
Autonomous nervous system
Cardiovascular parameters
Implantation surgery
Pig
Telemetry

ABSTRACT

The first aim of this study was to establish a surgical procedure to implant a new telemetric device for the continuous recording of electrocardiogram (ECG) and blood pressure (BP) in freely moving pigs. A second aim was the functional assessment of cardiovascular parameters, including heart rate variability (HRV) and blood pressure variability (BPV), so that these data could be used as the basis for the objective evaluation of autonomic activity and balance in different behavioural contexts. Eleven domestic pigs (German Landrace) underwent surgery for the placement of a telemetric device. At day 15 after surgery, 512 consecutive inter-beat intervals and pressure waves were analysed using different detection methods (automatic and manually corrected) while the animals were resting or feeding, respectively. HRV and BPV were calculated.

Incomplete datasets were found in four pigs due to missing ECG or BP signals. Technical and surgical issues concerning catheterisation and detachment of the negative ECG lead were continuously improved. In the remaining pigs, excellent signal quality (manually corrected data of 1%) was obtained during resting and acceptable signal quality (<10%) was obtained during feeding. Automatic triggering was sufficiently reliable to eliminate errors in BP recordings during active behaviour, but this was not the case for ECG recordings. Sympathetic arousal with accompanying vagal withdrawal during feeding was documented. The established surgical implantation and functional assessment of the telemetric system with the reliable registration of cardiovascular parameters in freely moving pigs could serve as a basis for future studies of autonomic regulation in context of stress and animal welfare.

Introduction

Advances in technology allow the use of mobile, invasive telemetric systems that are able to verify a number of cardiac parameters. Usually, telemetric systems find application in the context of pharmacological methods and cardiovascular diseases (Gelzer and Ball, 1997; Gross et al., 2002), and they have been used in animals such as rabbits (Van den Buuse and Malpas, 1997), monkeys (Chaves et al., 2006), rats (Beig et al., 2007), dogs (Ollerstam et al., 2007), miniature pigs (Stubhan et al., 2008) and domestic pigs (Poletto et al., 2011). Analysis of cardiac activity has also been used to investigate changes in sympathovagal balance related to emotional states (Désiré et al., 2004; Düpjan et al., 2011; Zebunke et al., 2011, 2013). Most studies that incorporate cardiac activity to evaluate subjective states in farm animals investigate the assessment of heart rate (HR) and its variability (HRV) by the use of non-invasive systems. Nevertheless, information about autonomic regulation obtained by these parameters is limited, as they provide information about the combined activity of both branches of the autonomic nervous system or about parasympathetic activation alone (von Borell et al., 2007). The assessment of blood pressure (BP) and its variability (BPV) is one possible approach to augment information about autonomic regulation, as the fluctuations in BP are indicative of sympathetic control (Hedman et al., 1992). Fully implantable telemetric techniques enable the measurement of electrocardiogram (ECG) and BP simultaneously while physiological variables are precisely assessed with minimal disturbance to the animal. Measurements can be realised in complex experimental designs, as animals can range freely in groups without restraint or contact by a handler.

The first aim of this study was the establishment of a surgical implantation method in freely moving pigs for a telemetric device used for the continuous recording of ECG and BP. The second aim

* Corresponding authors. Tel.: +49 38208 68800.
E-mail addresses: langbein@fbn-dummerstorf.de (J. Langbein); puppe@fbn-dummerstorf.de (B. Puppe).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tvjl.2015.10.050
1090-0233/

was the functional assessment of detected parameters to calculate HRV and BPV in order to enable objective evaluations about autonomic responses in different behavioural contexts. This may serve as a basis for future studies concerning autonomic control during situational appraisal in the context of stress and animal welfare.

Materials and methods

Ethical statement

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-037/12).

Animals

Eleven female domestic pigs (*Sus scrofa*, German Landrace) obtained from the Leibniz Institute for Farm Animal Biology in Dummerstorf (FBN) were used. At the beginning of their 10th week of life, piglets were transferred to an experimental barn and kept in individual pens (2.67 m^2–3.31 m^2). They had free access to water and were fed twice a day. After a 1-week acclimatisation period, the pigs were weighed and they underwent a physical examination. Once bodyweight exceeded 30 kg they became suitable for surgery, as arterial diameter had to be sufficient to insert and advance the catheter inside the vessel without resistance.

Telemetry equipment and signal monitoring

The implantable transmitter unit (Telemeter model TRM84PB, Telemetry Research) weighed 64 g and measured 90 × 45 × 10.5 mm. Two flexible bio-potential leads (30 cm in length) and a fluid-filled catheter (1 mm OD [3 Fr] Catheter, Millar Instruments) extended from the body of the transmitter. Using an individual frequency, each implanted transmitter relayed digital signals to a receiver unit attached at a 2.42 m height in front of each single housing pen. A switch at the receiver enabled the transmitter to be turned on and off in vivo without affecting the animal. The receivers were wire connected to two digital data acquisition systems (PowerLab 16/35 and 4/35, ADInstruments) that allowed simultaneous real-time recording of up to six pigs at a sampling rate of 2 kHz. The sampled data were stored and displayed on a computer and analysed using LabChart Pro (Version 7.0, ADInstruments).

Surgical procedure for transmitter implantation

Pigs were fasted 12 h prior to surgery but allowed access to water. They were anaesthetised IM in their home pen with a combination of xylazine 2 mg/kg (Xylariem, Riemser Arzneimittel) and ketamine 20 mg/kg (Ursotamin, Serumwerk). For local anaesthesia, procaine 4 mg/kg (Isocain, Selectavet, Dr. Otto-Fischer) was injected SC according to the planned incision lines. During surgery, general anaesthesia was maintained using IV ketamine 4 mg/kg/h (Ursotamin, Serumwerk) and diazepam 0.4 mg/kg/h (Faustan, AWD-pharma) in a 5% glucose solution. The implant was placed into a SC pouch formed on the left side of the neck. The catheter and the negative electrode were tunnelled SC to the ventral aspect of the neck using a hollow trocar. The left carotid artery was occluded with an arterial clamp, and a small incision was made to carefully insert the tip of the catheter into the vessel. Depending on the size of the pig, the catheter was advanced stepwise 7–10 cm to a point proximal to the aortic arch until a strong BP signal with characteristic slope was observed using the software. Five non-absorbable sutures (Dermafil, green polyester, USP 2/0 EP3) were placed to secure the catheter at the arteriotomy site. The negative electrode was tunnelled caudal to a small incision (1 cm) lateral to the sternum. The positive electrode was tunnelled caudal from the SC pouch to a small incision approximately 2 cm lateral to the left scapula. The tips of both electrodes were enclosed by muscle tissue and secured with circumferential ligatures of non-absorbable suture (Dermafil, green polyester, USP 2/0 EP3). Adequate ECG signals were verified. The transmitter body enclosed with surgical mesh was fixed to the surrounding tissue (Vitafil white, USP 2/0 EP3). To suture the dermis (subcutis), absorbable material was used (Ethicon, Vicryl plus 2-0, violet). Incisions in the epidermis were closed with a non-absorbable suture (Vitafil white USP 3 and 4 EP6). The locations of the components of the telemetric device inside the animal are shown schematically in Fig. 1.

Pigs were administered a peri-operative dose of metamizole 50 mg/kg (Metapyrin, Serumwerk Bernburg) and a combination of sulfadimidine 20 mg/kg and trimethoprim 4 mg/kg (Trimethosel, Selectavet, Otto-Fischer). This regimen was repeated every 24 h over 5 days post-surgery. Pigs were continuously observed in their home pens, and ECG and BP were monitored until the pigs exhibited complete recovery from anaesthesia. Surgical sites were examined, cleaned and treated with skin protection lotion (ImmuStim, almapharm) four times daily until the incisions were fully healed. Skin sutures were removed on postoperative days 10 and 11. Depending on battery life, transmitters were explanted between 30 and 60 days after surgical implantation. Pigs were sedated with a combination of xylazine 2 mg/kg (Xylariem) and ketamine 20 mg/kg (Ursotamin) prior to being euthanased by IV injection of tetracaine hydrochloride 0.5 mg/kg, mebezonium iodide 5 mg/kg and embutramide 20 mg/kg (T61, Intervet International). During post-mortem examination, transmitter body, ECG leads and BP catheter were verified to have remained in place before transmitters were explanted.

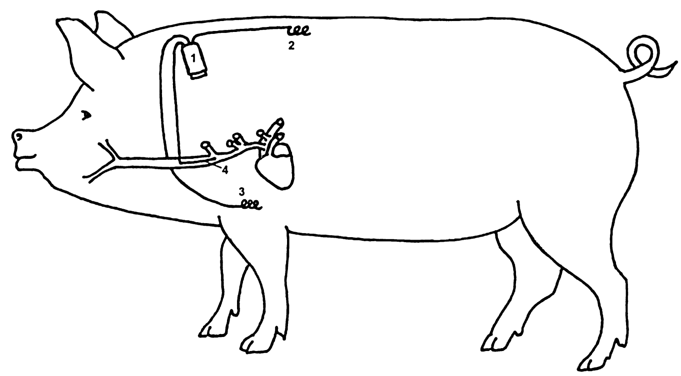

Fig. 1. The location of the components of the telemetric device inside the animal: (1) the transmitter body at the left side of the neck; (2) the positive electrode on the dorsal side lateral to the left scapula; (3) the negative electrode on the ventral side lateral to the sternum; and (4) the pressure catheter in the carotid artery.

Data acquisition and analysis

Behaviour, ECG and BP were monitored for 30 days. For the present study, data acquisition was performed on day 15 after surgery, which provided sufficient time for recovery from surgery, wound healing and examination of the compatibility of the device in the animal before data collection commenced. A familiar person entered the experimental room at 08.15 a.m. daily to feed the animals. Both behaviour (resting, feeding) and physiological responses (ECG and BP) were continuously recorded for 2 h pre-feeding and during food intake. The behaviour was videotaped. Cardiovascular data from the pre-feeding period when the pigs were lying inactive (resting) and during the consumption of food (feeding) were chosen. Segments of ECG and BP data, 512 beats in length (Poletto et al., 2011), were selected for each pig during resting and feeding. Analysis of ECG and BP data was performed using two detection methods: (1) automatic detection of valid QRS complexes and systolic pressure waves using standard software settings after applying a 50 Hz low-pass filter (treatment group: AUTO), and (2) automatic triggered points were manually checked for correct marking. If markings of a valid QRS complex or systolic pressure wave were missing or incorrectly set, they were manually adjusted. If a QRS complex or systolic pressure wave was completely missing, it was corrected by interpolation calculated from the mean of three previous and three subsequent values (treatment group: CORR).

HRV was calculated on the basis of interbeat intervals (IBIs) derived from the ECG signal. For the time domain, mean heart rate (HR), the standard deviation of IBIs (SDNN, an indicator of sympathetic and parasympathetic activation), the root mean of the squared distances of subsequent IBIs (RMSSD, an indicator of parasympathetic activation), and the ratio of these two (RMSSD/SDNN, reflecting the balance of the autonomous nervous system) were computed. Fast Fourier Transformation (FFT) was performed to obtain the power spectrum of the HRV data segments (LabChart). According to Poletto et al. (2011), a Hanning window was applied to each data set (FFT size, 512) to calculate the power in the low frequency band (LF, 0.0–0.09 Hz) and the high frequency band (HF, 0.09–2.0 Hz) in absolute units (ms^2), and the LF:HF power content ratio from the recordings. These parameters were assumed to be highly correlated with those of the time domain. The LF indicates sympathetic and vagal activation (analogically to SDNN), while the HF component correlates with RMSSD and is considered as a marker of vagal activation (Akselrod et al., 1981). Using the BP signal, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure ($MAP = DBP + 1/3 \times (SBP - DBP)$), and their standard deviations were assessed (standard deviation of systolic blood pressure [SDS], of diastolic [SDD] and of mean arterial pressure [SDM]). Analysis of BPV in the frequency domain was not performed in this case, as power spectral analysis in LabChart was not designed for BPV analyses.

Statistical analyses

The normality of distribution of each parameter was assessed using Kolmogorov–Smirnov tests. Where normality assumptions were not met, data were logarithmically transformed. All statistical analyses were conducted using SAS (version 9.3, 2009, SAS Institute). The differences in the number of corrected values between behavioural categories (resting, feeding) were determined. One-way analysis of variance (ANOVA, Glimmix procedure) was performed for IBI, SBP and DBP, with behaviour as a fixed factor. Additionally, 512-beat intervals with continuous data series of IBI, SBP and DBP were analysed using a two-way analysis of variance (ANOVA, Glimmix). The