U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER
ABERDEEN PROVING GROUND, MD 21010-5424

DEVCOM CBC-TR-1848

Development of a Multifaceted Telemetry System for Physiological Data Collection in Ferrets

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RESEARCH AND OPERATIONS DIRECTORATE

May 2023

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REPORT DOCUMENTATION PAGE

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED	
XX-05-2023	Final	Mar 2020	END DATE Dec 2020

4. TITLE AND SUBTITLE
Development of a Multifaceted Telemetry System for Physiological Data Collection in Ferrets

5a. CONTRACT NUMBER	5b. GRANT NUMBER	5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER	5e. TASK NUMBER	5f. WORK UNIT NUMBER
CB10704		

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, DEVCOM CBC, ATTN: FCDD-CBR-TO, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
DEVCOM CBC TR-1848

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)	11. SPONSOR/MONITOR'S REPORT NUMBER(S)
Defense Threat Reduction Agency, 8725 John J. Kingman Road, MSC 6201, Fort Belvoir, VA 22060-6201	DTRA	

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Statement A. Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT (LESS THAN 200 WORDS)
When investigating pathophysiological responses to novel chemical toxicants, describing homeostasis of major vital organ systems is critical to understanding host–toxicant interaction. In this study, we developed a ferret model to collect data that describe the cardiovascular (electrocardiogram and blood pressure), central nervous system (electroencephalogram), and respiratory system responses (diaphragmatic electromyogram, dEMG) as well as body temperature and animal activity. In addition, a jugular vein cannula attached to a vascular access button was inserted in each ferret for collecting serial blood samples. We used two systems of telemetry units. The first system consisted of one each of HD-X02 and HD-S11-F2 devices. This system was found to be less than ideal. The second system consisted of one each of F50-EEE and HD-S1-F2 devices. It was found to be more compatible with the regular housing and experimental conditions utilized for ferrets. A total of 18 ferrets were tested; 17 of them survived the surgical procedures. Herein we detail the optimized surgical procedures and housing arrangements and provide example data acquired from the implanted animals. Following this study, a more in-depth investigation into the utility of the dEMG for derivation of volume-related respiratory parameters will be conducted.

15. SUBJECT TERMS
Ferret, Electrocardiogram, Blood pressure, Electroencephalogram, Jugular vein catheter, Diaphragmatic electromyogram

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
a. REPORT	b. ABSTRACT	C. THIS PAGE		
U	U	U	UU	28

19a. NAME OF RESPONSIBLE PERSON	19b. PHONE NUMBER *(Include area code)*
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Prescribed by ANSI Std. Z39.18

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PREFACE

The work described in this report was authorized under project number CB10704. The work was started in March 2020 and completed in December 2020. This work was performed at the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD).

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CONTENTS

FIGURES

DEVELOPMENT OF A MULTIFACETED TELEMETRY SYSTEM FOR PHYSIOLOGICAL DATA COLLECTION IN FERRETS

1. INTRODUCTION

Monitoring *in vivo* biopotentials with implantable devices that transmit data through radio frequency is not a new concept. One of the earliest patents of this technology, a device capable of sensing and transmitting a biopotential between two electrodes, was granted in 1965[1]. Beginning around this time, experimentation occurred mainly in large animals, monitoring blood pressure (BP)[2] and the electrocardiogram (ECG)[3]. Advances in the capabilities of telemetric transmitter technologies resulted in the ability to monitor multiple biopotentials simultaneously. In 1974, Pauley et al. described a device capable of collecting electrooculargram, electromyogram (EMG), ECG, and three channels of electroencephalogram (EEG) in the pigtail macaque[4]. Since then, numerous academic and private investigators have made advancements in both surgical techniques and telemetry technology. The use of implantable devices in *in vivo* physiological studies has increased to the point that it is now commonplace in academic, pharmaceutical, and government research programs. The current trend is moving towards acquisition of data that describe the effects of a drug, toxicant, or infectious agent on multiple vital organ systems[5,6,7,8]. As a result of device size limitations, often times, multiple telemetry units must be implanted to acquire all desired endpoints.

2. BACKGROUND

2.1 Considerations When Implanting Multiple Telemetry Devices

The advantages of implanting multiple telemetry channels and/or units are rooted in two of the three R's of animal research; reduction of animal use and refinement of experimental methods. Animal use is reduced in studies requiring multiple physiological endpoints through the elimination of satellite groups. Satellite groups are required for studies in which data from multiple physiological systems is necessary but cannot be simultaneously derived from the same animal. This study design requires not only twice the number of animals, but is also subject to dosing errors and inter-animal variability, reducing the overall statistical power of the study. By implanting a single animal with sensors for all required physiological endpoints, a study is able to be conducted on a single group, for which each animal serves as its own control. The result is a refined study providing a more complete picture of the physiologic responses to a test compound.

The difficulty associated with implanting two telemetry devices arises from the extensive surgical procedures required to place the sensors, as well as choosing a system of transmitters and receivers that will allow for collection of all required endpoints without frequency interference. Furthermore, as the animal species become physically smaller, the body burden of the implanted system becomes greater. In animal species weighing more than 2 kg, collecting ECG, BP, body temperature (BT) and respiratory function through impedance pneumography can be accomplished with a single implantable device 9,10,11.

Collecting the same data from animals under 1.5 kg requires the implantation of two smaller devices.

We have previously implanted rabbits with a multi sensor system to monitor the ECG, BP, impedance based tidal volume (Vt), BT and physical activity (ACT)[8]. That model was later modified to include a second telemeter to monitor the EEG[12]. The benefit of this model is simultaneous monitoring of all major vital organ systems in an unrestrained animal. Current research within this laboratory is focused on studies for which the ferret is the preferred animal model. Therefore, in the present study, a ferret model was developed in which telemetric monitoring of the ECG, EEG, diaphragmatic electromyogram (dEMG), BP, BT, and ACT was accomplished, with the addition of a central venous blood sampling port. From this model a host of physiological parameters can be derived. Combining the physiologic responses with clinical blood chemistry and hematology outcomes results in a more detailed understanding of the major physiological impacts of a chemical toxicant.

3. MATERIALS AND METHODS

3.1 Surgical Supplies

All telemetry units, HD-S1-F2, HD-S11-F2, F50-EEE, and HD-X02, as well as data collection & processing software, Ponemah V 6.12, were purchased from Data Sciences International (DSI; St. Paul, MN). Rat jugular vein catheters, rat single-channel Vascular Access Buttons™ (VAB), and PinPort™ injectors were ordered from Instech Laboratories Inc. (Plymouth Meeting, PA). Catheters were ordered with the following specifications: 3Fr external diameter, 0.64mm internal diameter medical grade polyurethane 20cm in length with silicone collars at 2.5cm and 2.8cm, fitting a 22ga insert. Suture, 4-0 Ethibond Excel and 4-0 Vicryl were purchased from Ethicon (Somerville, NJ). DuraPrep™ surgical solution (iodine povacrylex and isopropyl alcohol, 74% w/w) was purchased from 3M (St. Paul, MN). Taurolidine-citrate solution (TCS) was purchased from Access Technologies (Skokie, IL). All other surgical medications and chemicals were purchased from Patterson Veterinary Supply, Inc. (Devens, MA). Microtainer® EDTA-coated blood collection tubes were purchased from Becton Dickinson (Franklin Lakes, NJ).

3.2 Animal Model

An animal use protocol was reviewed and approved by the Combat Capabilities and Development Command, Chemical Biological Centers' Institutional Animal Care and Use Committee, adhering to the latest version of the Guide for the Care and Use of Laboratory Animals. Ten castrated and descented male ferrets (*Mustela putorius furo)* weighing 0.9 – 1.2kg, were purchased from Marshall BioResources (North Rose, NY). Animals were housed in an AAALAC-accredited facility in groups of four in double-wide, rabbit cages (10.4 ft^2) modified to prevent escape (Lenderking Caging Products, Millersville, MD). Food (High Density Ferret Diet; LabDiet, St. Louis, MO) and water were provided *ad libitum*. Temperature and humidity were maintained between 68˚F-72˚F (20˚C-22˚C) and 30%-70% relative humidity with a

light/dark cycle of 12h/12h. Ferrets were provided with a five day acclimation period before use on study.

3.3 Surgical Procedure

During the development of this model several transmitter types were utilized and therefore slight differences in the surgical approach were employed. The procedure described below represents the surgical approach of the optimized model.

3.4 Pre-surgical Considerations

Prior to surgery, ferrets were fasted, 2-4 hours, to minimize stimulation of the emetic reflex upon administration of anesthesia. Anesthesia was induced by placing the ferret in a charged induction chamber of 4% isoflurane and medical grade O_2 at 2.0 L/min for five minutes. Following induction, the ferret was fitted with a non-rebreathing mask, and general anesthesia was maintained with isoflurane between 2-3% delivered in medical grade O_2 at 1 L/min. Eye lubricant was applied to both eyes and buprenorphine (0.03 mg/kg SQ), atropine (0.04 mg/kg SQ), and cefazolin (20 mg/kg, IM) were administered pre-operatively. The surgical fields were scrubbed and prepped with 4% chlorhexidine solution, 70% isopropyl alcohol, and DuraPrep™. The absence of palpebral and pedal reflexes was confirmed prior to initial incision and a surgical plane of anesthesia and aseptic techniques were maintained throughout the entire surgical procedure.

3.5 Detailed Procedure

With the animal placed in dorsal recumbency, a laparotomy was made along the *linea alba* extending cranially to the xyphoid process and caudally, to the navel. The F50-EEE was partially tacked at the caudal aspect of the abdominal incision and sterile gauze, dampened with warm sterile saline was used to cover the incision. The ferret was placed in ventral recumbency and a 1.5-2.0 cm incision was made between the scapulae. The EEG leads were tunneled from the abdominal midline to the scapulae incision by momentarily placing the animal in left lateral recumbency before being replaced in ventral recumbency. A 2.5-3.0 cm, long incision was made on the top of the head along the sagittal crest from the occipital crest to the frontal bone and the muscle was retracted from the sagittal crest. Two craniotomies were made, using a #56 drill bit (Plastics One, Roanoke VA) for the positive and negative EEG electrodes using the occipital crest as a landmark. The craniotomy for the negative electrode was made 10 mm anterior to the occipital crest and 3 mm to the right of the sagittal crest. The craniotomy for the positive electrode was made 25 mm anterior to the occipital crest and 3 mm to the left of the sagittal crest (Figure 1). Screws 0.080” x 0.125” (Plastics One; Roanoke, VA) were placed into each craniotomy and the EEG electrodes were wrapped around the screw as it was tightened into place. Dental acrylic was used to insulate the connection from fluid infiltration. The muscle and skin were closed using 4-0 vicryl suture. Sterile gauze was placed over the scapular incision and the animal was then placed in dorsal recumbency.

From the laparotomy, the left side of diaphragm was located and the two dEMG electrodes were inserted perpendicular to the muscle fibers 3-5 mm apart (Figure 2). Placement was confirmed using acquisition software to monitor respiration and the electrodes were secured

in place with 4-0 ethibond suture. The grounding electrode was secured to the left side of the abdominal muscle with 4-0 ethibond suture. The ECG leads were tunneled from the abdomen and placed in modified lead II configuration (intravenous negative electrode). The negative electrode was tunneled to an incision made at the right jugular groove. The positive electrode was tunneled to the left chest muscle, 2.0 cm lateral to the xyphoid process and secured in place with 4-0 ethibond suture. The abdominal muscle was closed with 4-0 vicryl suture securing the F50-EEE device along the suture line. The laparotomy was extended 4.0 cm caudally to accommodate the HD-S1-F2 unit. A 1.5 cm incision, was made at the right femoral groove and the BP catheter was tunneled from the laparotomy to the femoral incision. The femoral artery was located, isolated, ligated, and the catheter was introduced. The catheter was advanced into the artery, 3.5-4.0 cm, and placement was confirmed using acquisition software before securing in place with 4-0 ethibond suture. All muscle and skin incisions were closed with 4-0 vicryl.

While the skin was being closed, the JVC, was implanted as previously described[13]. Once the JVC was in place, the negative ECG electrode was introduced, 0.3-0.4 cm, adjacent to the JVC. Both were secured with 4-0 ethibond after confirmation of negative ECG placement using acquisition software. All incisions were infiltrated with ropivacaine and 3 mg/kg carprofen was administered SQ.

3.6 Post-surgical Considerations

Following surgery ferrets were placed in a recovery cage until ambulatory and were then returned to single housing for 24 hours before returning to group housing. Post-operative pain management was accomplished using buprenorphine (0.03 mg/kg SQ, BID) and carprofen (3 mg/kg SQ SID) for up to three days based on a recovery scoring protocol.

3.7 Data Acquisition Array

Data were acquired using the Ponemah Physiology Platform version 6.12 (Data Sciences International; Minneapolis, MN) and RPC-3 receivers. The receivers were arrayed under each cage so as to maximize receiving area. Animals were housed within a rack to avoid crosstalk between implants when animals move towards the edges of a cage. Hanging and elevated enrichment was moved to the floor during data recording.

4. RESULTS

4.1 Surgical Outcomes

A total of eighteen ferrets were implanted with a two-telemetry unit system, with or without a JVC and VAB. Four ferrets were implanted with the HD-S11-F2/HD-X02 and JVC/VAB combination (system one) and six were implanted with the HD-S1-F2/F50-EEE and JVC/VAB (system two). Eight additional ferrets were implanted with system two excluding the JVC/VAB (not required for the study). There were several disadvantages associated with system one that were corrected with system two.

All four ferrets implanted with system one survived the surgical procedure. Two JVCs in this group lost patency and all four HD-X02 devices experienced significant dropout during data

collection with unexpected, rapid loss of battery life. Thirteen of the fourteen ferrets implanted with system two survived. One ferret experienced anesthetic complications during the procedure and did not recover. The data collected from system two had minimal drop out, but cross talk between transmitters and battery life of the devices were significant issues. In three of the fourteen surviving ferrets assigned to system two, the blood pressure signal was lost between six and 8 days following surgery. It was later determined that this was due to kinking of the blood pressure catheter. Radiographs of the completed procedures for system one and system two are shown in figures 3 and 4 respectively. Example data from one animal implanted with system two is shown in Figures 5 and 6.

5. DISCUSSION

Our laboratory is often tasked with characterizing the physiological response to chemical toxicants that have never before been tested *in vivo*. A critical aspect of these studies is gathering as much physiological data as possible to clearly define the pathophysiology of intoxication. As the animal species decrease in physical size, collection of this data becomes more difficult without the use of satellite groups. The goal of this study was to develop a ferret model from which investigators can collect EEG, ECG, dEMG, BP, BT, ACT and venous blood samples from conscious free moving ferrets before, during, and after exposure to chemical toxicants. Development of this model leveraged existing small animal telemetry devices and methods for the collection of these parameters individually to combine and refine the surgical procedures.

Initially, the goal was to work with devices designed for smaller rodent species in order to minimize the body burden from implanting two devices and for translation of procedures developed in the ferret to smaller animal species. This system consisted of one DSI HD-X02 and one HD-S11-F2 transmitter and was designated system one. The HD-X02 was utilized to collect the dEMG and EEG; the HD-S11-F2 transmitter was utilized to collect the ECG, BP, BT and ACT. Four ferrets were implanted with system one and while surgery was successful, implementation of the total system and data collection were problematic in the ferret for several reasons.

The placement of the HD-X02 device in a scapular pocket behind the VAB resulted in two issues precluding further use of this device. The first being effective distance from the receiver array, the HD-X02 transmitter was being operated at the upper limits of its transmission range. Therefore, often times the animal would move vertically within its caging which would result in the frequent dropout observed in the data. The second issue with this placement was that the transmitter body migrated to a position under the VAB which contains a magnet to hold a protective cap in place. This magnet continuously turned the transmitter on and off resulting in dropout during recording, and usage of battery life when recording was not ongoing. Another issue was that the ECG was collected with a different transmitter than the dEMG. During analysis of the dEMG waveform, artifact from the R-wave of the ECG is removed based on time synchronization between the two waveforms. When these two data streams are collected with different transmitters the time offset is variable and results in difficulty removing the R-wave artifact. Finally, in two of the four ferrets in this group the VAB became non patent at days 3 and 4 post-surgery. During a second procedure to move the HD-X02 away from the VAB, it was discovered that the JVCs of the two non-patent animals were occluded by the EEG leads from

the HD-X02 unit. This issue would be corrected by routing the leads away from the JVC in system two procedures. The performance of system one could be optimized for use in a small animal model that is housed closer to the receiver array; however for the ferret, a transmitter with greater transmission power was required.

System two was developed to correct the issues that became apparent in system one. The HD-X-02 device was replaced with an F50-EEE and the HD-S11-F2 was replaced with an HD-S1-F2. The F50-EEE affords a longer transmission distance than the HD-X02 and provides all three biopotential lead sets, correcting the previous issue associated with ECG artifact removal during dEMG analysis. This device was implanted into the abdomen, placing it closer to the receiver array in three-dimensional space and moving it away from the magnet in the VAB. The HD-S1-F2 was used to collect BP data, and was placed directly caudal to the F50-EEE device. All animals that survived surgery (13 of 14) initially presented with high quality signals from all physiological systems. As time progressed, the blood pressure signal was dampened or lost in three of the fourteen ferrets. This was initially thought to be a result of the catheter backing out of its placement in the femoral artery. Upon necropsy, it became apparent that the issue was a result of the transmitter body tumbling in the SQ pocket resulting in kinking of the catheter. This tumbling is a result of the transmitters not being secured to the underlying muscle as they did not have suture tabs. Recent work using suture tabs to secure the HD-S1-F2 transmitter body along the laparotomy have mitigated this issue. Surprisingly, random signal dropout and random activation or deactivation of the transmitters was still problematic in animals implanted with the VAB. This was determined to result from the VAB of a cagemate coming close enough to the transmitter to turn it on or off. The use of VAB's without magnets or housing with cagemates not implanted with a VAB is required to completely ameliorate this issue.

In this study the surgical procedure, hardware selection and data acquisition method for the collection of EEG, ECG, dEMG, BP, BT, ACT and venous blood samples were optimized for use in the ferret. It was demonstrated that the first system is incompatible with the housing requirements for a ferret. This system should be optimal for smaller animal species with smaller housing requirements. The second system performed significantly better in the ferret yielding satisfactory data collection with the exception of loss of signal from three of the blood pressure catheters. The underlying cause of the signal loss was discovered and has been corrected. The final issue with this model was the ability of the magnet in the VAB to activate the on/off switch in the implanted telemetry units. This issue is simply resolved by either using VAB's without magnets, or by housing animals with a cagemate that does not have a VAB implanted. Continued work with this model will focus on derivation of respiratory rate and volume parameters from the dEMG, as well as translation of the procedures to smaller species such as the guinea pig and rat.

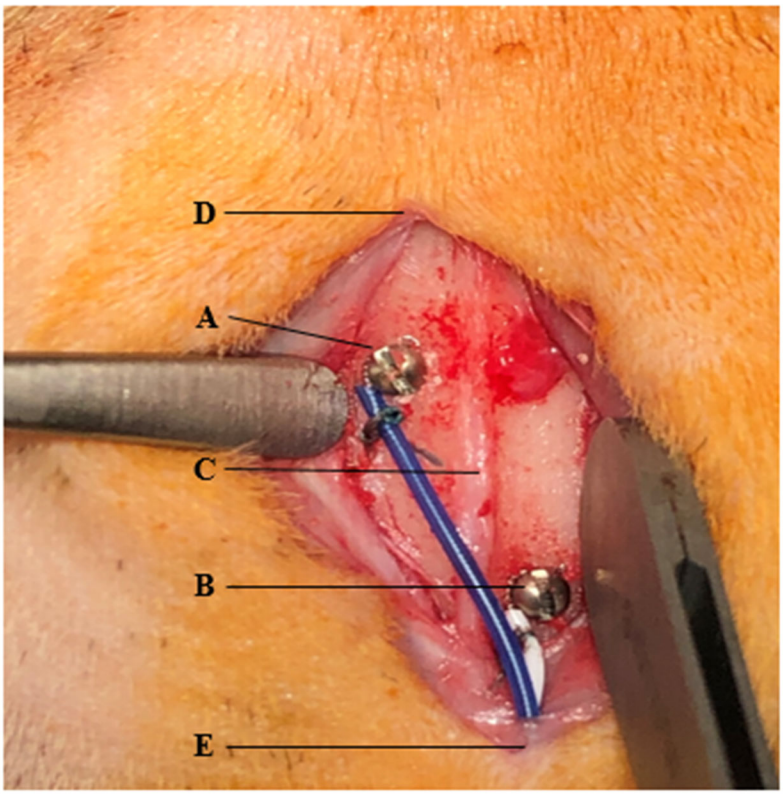

Figure 1. EEG Electrode Placement. Depiction of EEG electrode placement with positive electrode (A) and negative electrode (B) in relationship to the midsagittal crest (C), anterior aspect (D) and posterior aspect (E) of the skull incision.

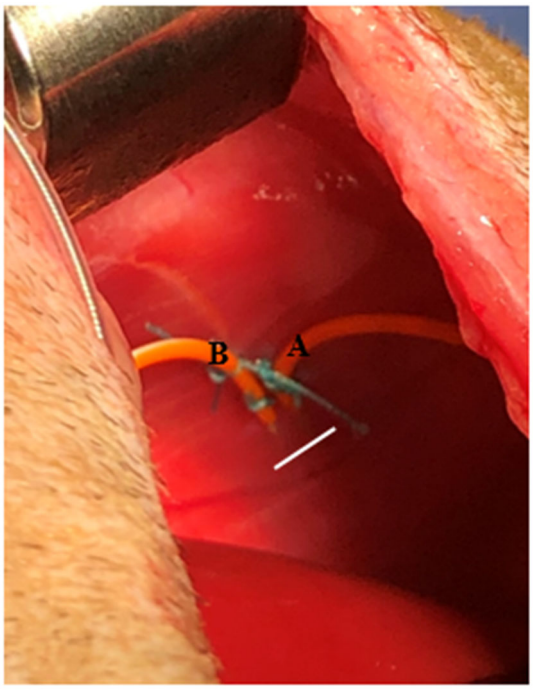

Figure 2. dEMG Electrode Placement. Depiction of EEG electrode placement with positive electrode (A) and negative electrode (B) placed perpendicular to the muscle fibers of the diaphragm. White line indicated terminus of exposed electrode, gap between electrodes is 2-3 mm.

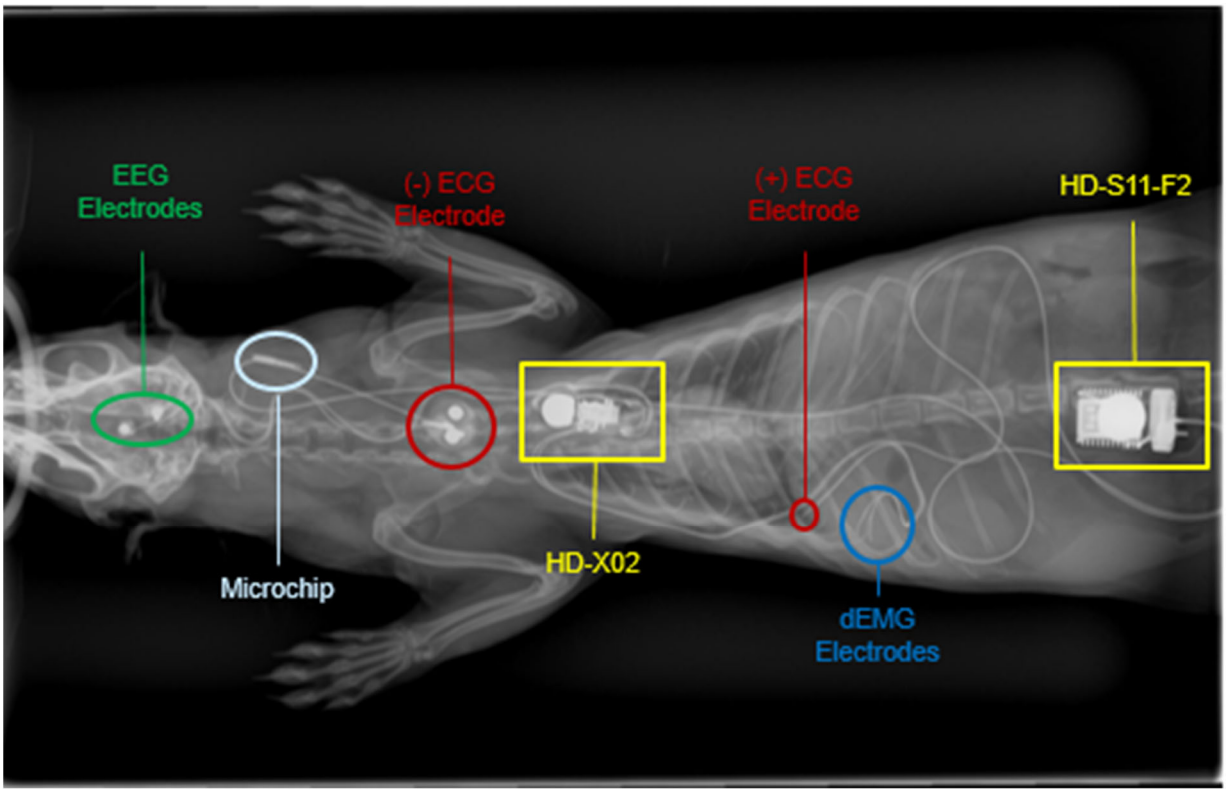

Figure 3. System One Dorsal Aspect. Location of transmitters and electrodes used in system one, the negative ECG electrode is placed in the jugular vein and appears here under the VAB.

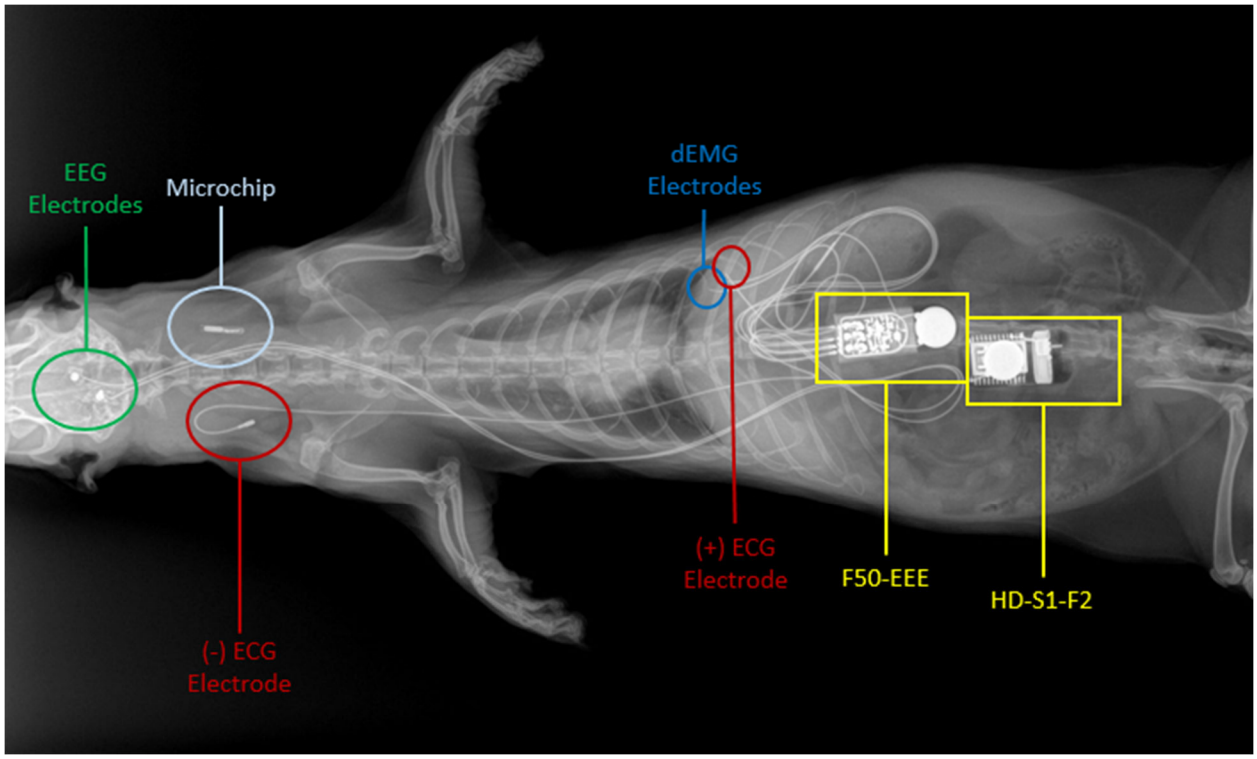

Figure 4. System Two Ventral Aspect. Transmitters are located along the midline abdominal incision and the negative ECG electrode is placed in the jugular vein. No VAB was placed in this animal.

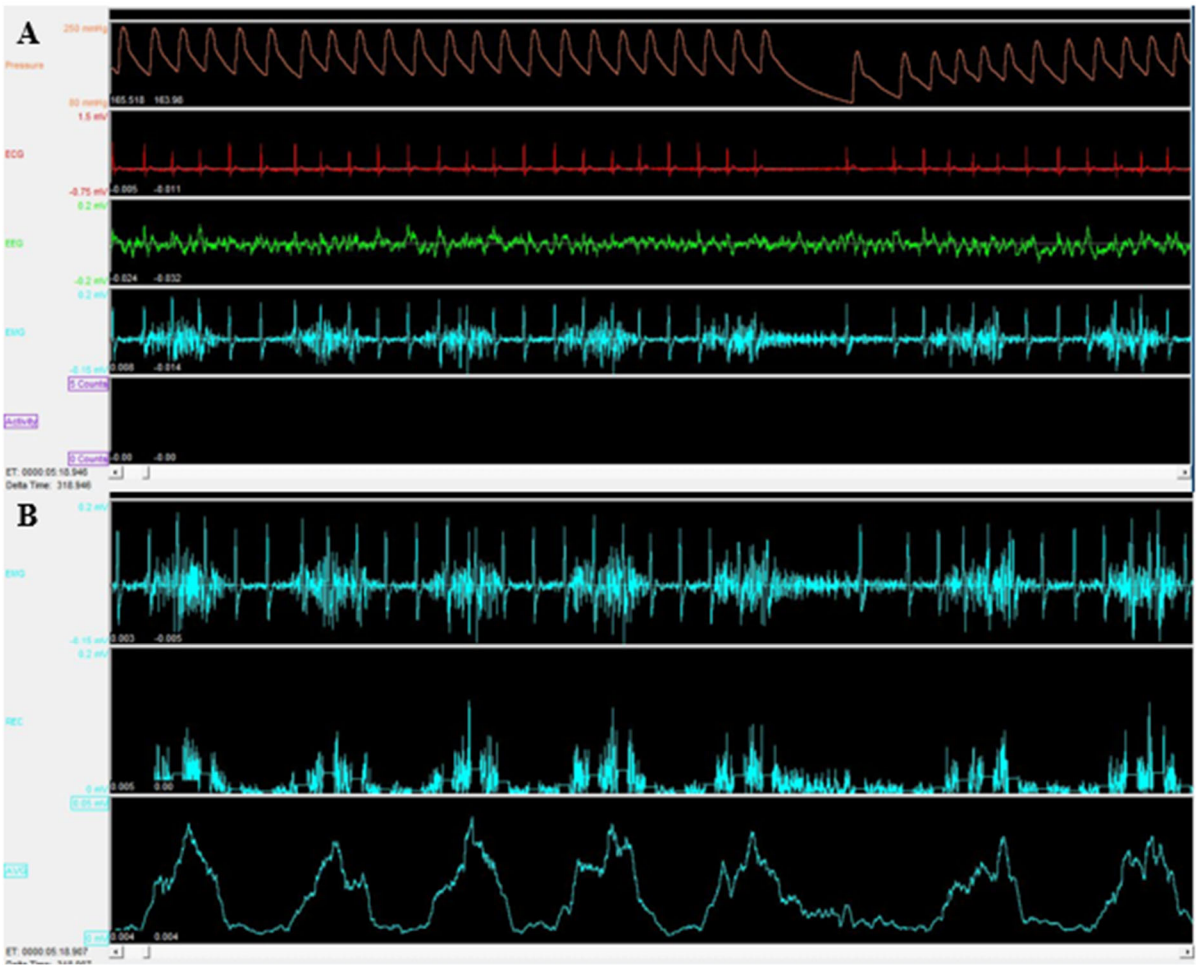

Figure 5. Resting Data. Example of data collected during a period of rest from a ferret implanted with system two. Panel A from top to bottom: BP, ECG, EEG, raw dEMG. Panel B from top to bottom: raw dEMG, rectified dEMG with R-wave ECG artifact removed, averaged rectified dEMG signal.

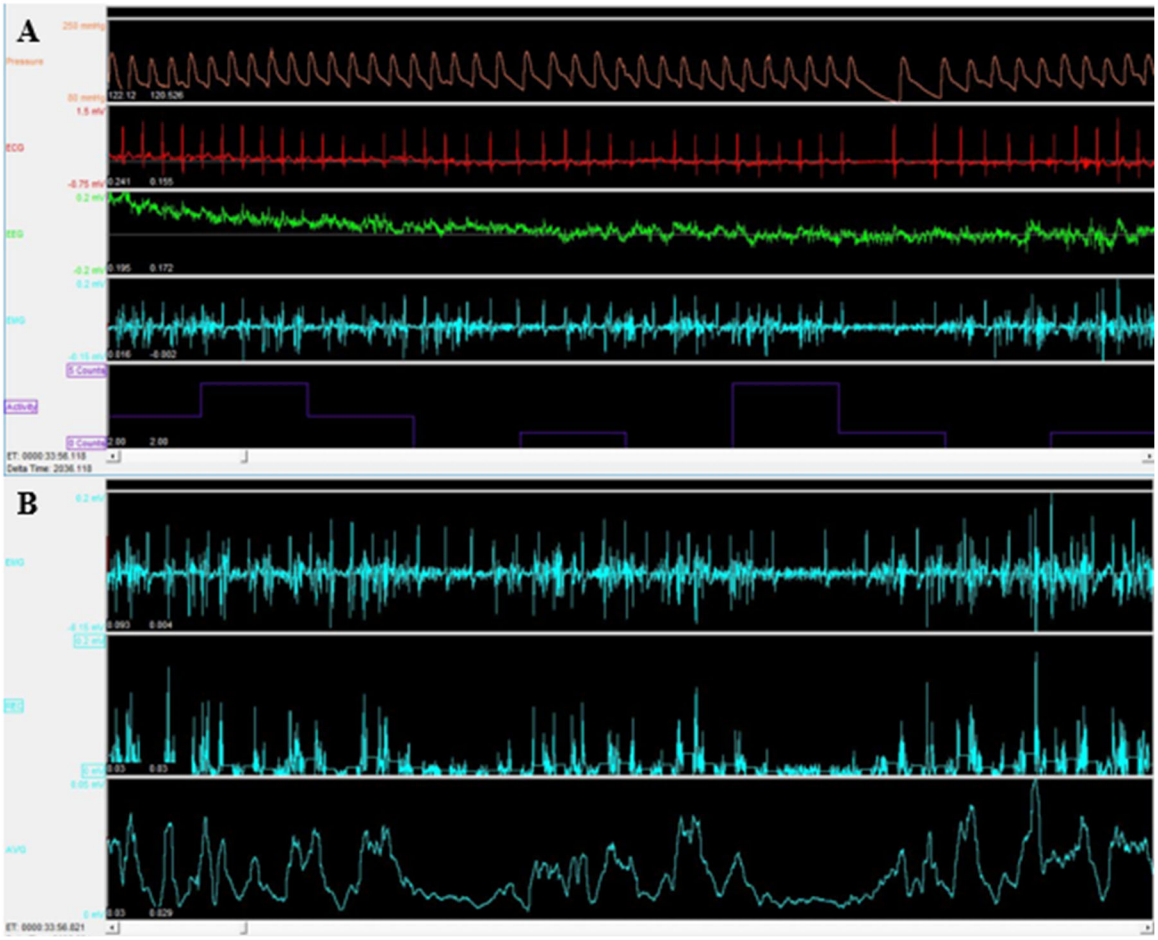

Figure 6. Active Data. Example of data collected during a period of activity from a ferret implanted with system two. Panel A from top to bottom: BP, ECG, EEG, raw dEMG. Panel B from top to bottom: raw dEMG, rectified dEMG with R-wave ECG artifact removed, averaged rectified dEMG signal.

LITERATURE CITED

1. Honig, William M. "Wireless passive biological telemetry system." U.S. Patent No. 3,218,638. 16 Nov. 1965.
2. Van Citters, Robert L., William S. Kemper, and Dean L. Franklin. "Blood pressure responses of wild giraffes studied by radio telemetry." *Science* 152.3720 (1966): 384-386.
3. Jahn, John Russell. "The use of electrocardiographic radio telemetry to determine heart rate in ruminants." (1963).
4. Pauley, J. Donald, Martin Reite, and Stephen D. Walker. "An implantable multi-channel biotelemetry system." Electroencephalography and clinical neurophysiology 37.2 (1974): 153-160.
5. Schierok, Hans, et al. "Continuous assessment of multiple vital physiological functions in conscious freely moving rats using telemetry and a plethysmography system." *Journal of pharmacological and toxicological methods* 43.3 (2000): 211-217.
6. Niemeyer, James E. "Telemetry for small animal physiology." *Lab animal* 45.7 (2016): 255-257.
7. Meindl, JAMES D., et al. "Implantable telemetry." *Methods of Animal Experimentation* 7 (2016): 37-112.
8. Horsmon, Michael S., et al. "An Impedance-Based Model for the Assessment of Cardiopulmonary Function in Rabbits." *Journal of the American Association for Laboratory Animal Science* 55.2 (2016): 213-220.
9. Kearney, Kenneth, et al. "Evaluation of respiratory function in freely moving Beagle dogs using implanted impedance technology." *Journal of pharmacological and toxicological methods* 62.2 (2010): 119-126.
10. Authier, S., et al. "Combined cardiopulmonary assessments with implantable telemetry device in conscious freely moving cynomolgus monkeys." *Journal of pharmacological and toxicological methods* 62.1 (2010): 6-11.
11. Willens, Scott, et al. "Novel technique for retroperitoneal implantation of telemetry transmitters for physiologic monitoring in Göttingen minipigs (Sus scrofa domesticus)." *Comparative medicine* 64.6 (2014): 464-470.
12. Horsmon M.S., et al. *Comprehensive Vital System Monitoring in the Rabbit*. Poster presented at the Annual Meeting of the Society of Toxicology March 2016.
13. Boeri, Chelsea R., and Michael S. Horsmon. "Implantation of Vascular Access Buttons™ for Prolonged Blood Collection in Venously-Catheterized Ferrets." *Journal of Investigative Surgery* (2019): 1-5.

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ACRONYMS AND ABBREVIATIONS

ACT	Animal activity
BP	Blood pressure
dEMG	Diaphragmatic Electromyogram
ECG	Electrocardiogram
EEG	Electroencephalogram
TEMP	Temperature

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APPENDIX I

Post-Surgical Monitoring Sheet

Ferret Post-Surgical Monitoring Log
Date of Procedure: _____ Animal #:_____
Procedure: _____
Post-Procedure Analgesia Regimen:

Drug	Dose (mg/kg)	Dose (mL)	Route	Frequency

Notes:

Monitoring (score each on scale of 1 - 3, with 1 = normal, 2 = requiring veterinary attention if persists for >2 days, 3 = requiring veterinary attention immediately):

Date/Time							
Activity							
Posture							
Grooming							
Incision							
Appetite							
Feces							
Analgesia Given?							
Assessment							
Weight (kg)							

Guidelines for scoring:
Activity: 1 = curious and active, moves freely and spontaneously about cage; 2 = indifferent or minimally curious about to human presence, moves about cage with encouragement; 3 = lethargic, does not get up even with encouragement
Posture: 1 = normal relaxed posture and gait; 2 = any abnormalities in posture, including but not limited to weight shifted to one side, hunched / reluctance to curl into normal position, stilted gait, shuffling gate; 3 = abnormal posture such as favoring one or more limbs, head tilt, falling over or rolling
Grooming: 1 = normal shiny smooth coat; 2 = mild ruffled or dull fur; 3 = marked matting, hair loss or ulceration / wounds
Incision: 1 = normal; 2 = swollen or red, if first noticed within 24 hours of surgery and not worsening; 3 = markedly swollen or red, discharge from site, bleeding from site, suture coming out / incision dehiscing
Appetite: 1 = normal appetite; 2 = decreased appetite / no appetite for chow but eats treats; 3 = no appetite for chow or treats
Feces: 1 = normal amount of normal feces; 2 = decreased amount of normal feces; 3 = no feces or diarrhea
Assessment: Score reflecting highest score assigned for above categories: 1 = normal, 2 = requiring veterinary attention if persists for >2 days, 3 = requiring veterinary attention immediately

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