ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)
Main Body
VERSION 4

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

A. **ACORP Status.**

1. Full Name of Principal Investigator(s)► [redacted]

2. VA Station Name (City) and 3-Digit Station Number► **McGuire VAMC Richmond, VA, Station #652**

3. Protocol Title► **A Comparison of Canine Anesthetic Regimens to Optimize Hemodynamic Stability and Quality of Electrophysiologic and Neurophysiologic Data Acquisition**

4. Animal Species covered by this ACORP► **Canines**

5. Funding Source(s). Check each source that applies:

►( ) Department of Veterans Affairs.
►( ) US Public Health Service (e.g. NIH).
►( ) Private or Charitable Foundation -- Identify the Foundation:
►( ) University Intramural Funds – Identify the University and Funding Component:
►( ) Private Company – Identify the Company:
►(X) Other – Identify Other Source(s): None

6. Related Documentation for IACUC reference.

a. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:

(1) Title of project►

If approved by the R&D Committee, give the date of approval►

b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

(1) Identify the studies described in the previously approved ACORP that have already been completed
►

(2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
►

(3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
►

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):

►(**X**) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

► Currently, there are several protocols using canines in research to study physiologic electrical impulses (electrophysiology) as it relates to cardiovascular diseases. An important part of these studies involve the acquisition of electrical impulse data from the heart in these animals during procedures which require sedation or general anesthesia. It has been well established that anesthetic agents can depress cardiac function and/or impair electrophysiological and neurophysiologic monitoring which may impact our ability to acquire accurate data. In order for these animal studies to produce genuine electrophysiological data that could later be relevant to human cardiovascular research, an optimal anesthetic regimen needs to be established. This study will investigate the impact of several different anesthetic regimens on dogs from pre-approved protocols (Protocol # 02243 and Protocol # 02235) and will therefore not require independent animals for this study. This will greatly reduce the loss of animal life for research. Anesthesia is an important element in animal research since it aims to reduce harm and discomfort for the animal, and this protocol will provide information for future research studies to provide the best care for canines undergoing surgery with sedation or general anesthesia.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

► The aim of this study is to identify the anesthetic regimen that will best optimize cardiovascular stability and the collection of neurophysiologic/autonomic and electrophysiological measurements in the canine model. It has been well established that

anesthetic agents can depress cardiac function and/or impair electrophysiologic nerve monitoring which may impact the ability to acquire accurate data. There are several canine research models which study cardiovascular disease and require surgery and anesthesia in order to place electrophysiologic and neurophysiologic monitors. Once placed, these monitors are used to track and record physiologic activity. This study will investigate the impact of several different anesthetic regimens as it affects neurophysiologic and electrophysiological recordings from these devices .

Study subjects will be transferred to our study from protocols # 02243 and/or # 02235 a minimum of ten days following surgical thoracotomy for implantation of a pacemaker andneurophysiologic and electrophysiologic recording devices. After 10 days animals will be evaluated for transfer to our study. The parameters assessed will include return of baseline function and activity; normal eating, sleeping and elimination behaviors; and minimal or no requirement for pain control medications, Additionally, the canine will need to have no weight loss for at least 3 days prior to transfer. Once transferred to our study, animals will be exposed to each of the anesthetic regimens as outlined below. The primary agents that will be investigated are brevital, isoflurane, propofol alone, propofol and ketamine, and alfaxalone. A minimum of 2 days will separate each anesthetic exposure in order to allow for adequate recovery, return of baseline level of function and clearance of anesthetic medications (based on estimated elimination half-life). A minimum of 2 days will separate each anesthetic exposure in order to allow for adequate recovery return of baseline level of function and clearance of anesthetic medications (based on estimated elimination half-life). The times the animal is sternal, standing, eating, drinking and producing urine will be documented on the surgical form. We will require at least 36 hours of normal behavior as observed prior to the anesthesia (normal activity, normal eating/drinking, normal stool/urine output) before continuing with the next regime. This would assume it should take less than 12 hours to fully recover from all anesthesia regimens. Should the animal show any signs of nausea (drooling/vomiting/anorexia), anti-emetics (Famotidine 0.5-1 mg/kg PO or IV or Metoclopramide 0.2-0.5 mg/kg IV) will be started to treat the symptoms. If these symptoms last longer than 48 hours, the animal will be removed from the study and not be given any further anesthetics on this protocol. Additionally, the canine will be weighed at least twice weekly while on this protocol and if the weight changes by 5%, daily weights will be taken. If weight drops by 10% or more, the animal will be removed from the protocol.
Following completion of our study, animals will be transferred back to Protocol # 02243 or Protocol # 02235.

2. **Complete description of the proposed use of animals.** Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.
►

In order to evaluate the effects of anesthetic agents on cardiac conduction, hemodynamic stability, and electrophysiologic and neurophysiologic signal monitoring, the following anesthetic regimens will be utilized as outlined below.

Anesthesia allows the provision of mild sedation to deep general anesthesia, depending on the nature and requirements of the procedure. For example, more stimulating and invasive procedures such as thoracotomy, pacemaker implantation, percutaneous myocardial biopsy, etc require general anesthesia and endotracheal intubation, whereas cardiac monitoring, electrocardiography and echocardiography generally only require sedation.

In our study, no actual surgical procedure will be performed. All study subjects will have implanted monitoring devices (such as a cardiac pacemaker and stellate ganglion and

vagus nerve monitors) placed as part of Protocols #02243 and #02235, and will thus already be in place for our study. Once deemed fit for transfer to our study (as described above), they will be randomly assigned to be exposed to each of the eight anesthetic regimens [4 General Anesthesia (GETA) and 4 Sedation (Sed) protocols] as outlined below for 30 minutes each.

ANESTHETIC REGIMENS:

All currently used anesthetics have been shown to interfere with normal cardiac conduction and/or vascular responsiveness to hemodynamic perturbations. Therefore, the purpose of the proposed study is to directly compare commonly used and available anesthetic agents and identify the optimal regimen.

In veterinary anesthesia, Brevital is a well-known induction agent, and isoflurane is commonly used for maintenance of general anesthesia (see Regimen GETA 1 below). For comparison, we have included the commonly used agents for human anesthesia, propofol and ketamine, titrated for canine requirements. We have also included alfaxalone, a newer and promising canine anesthetic. In order to maintain consistency with common veterinary practices, we have included the use of the analgesic, buprenorphine, a commonly used medication for surgical pain control.

General anesthetic (GETA) regimens including endotracheal intubation will be as outlined below: (As described above, these regimens would be used for more stimulating and invasive procedures such as thoracotomy, pacemaker placement, percutaneous myocardial biopsy, etc which require general anesthesia and endotracheal intubation. These surgical procedures will NOT be conducted on this protocol, only the anesthesia and intubation)

1) **GETA Regimen 1 (GETA 1) – each subject will receive the extensively studied and widely used standard protocol including premedication with Buprenorphine 0.01-0.02 mg/kg IM, followed by induction with Brevital 6-10 mg/kg IV bolus titrated to effect, to achieve a surgical plane of anesthesia, followed by endotracheal intubation and mechanical ventilation. Inhaled 1-4% isoflurane will be titrated for anesthetic maintenance for 30 minutes, after which the subject will be allowed to recover and will be extubated as soon as appropriate.**
2) **GETA Regimen 2 (GETA 2) – each subject will be premedicated with Buprenorphine 0.01-0.02 mg/kg IM, followed by 1% propofol 2.0-7.5 mg/kg IV bolus to achieve a surgical plane of anesthesia followed by endotracheal intubation and mechanical ventilation. Propofol infusion (10 mg/mL at 50-300 mcg/kg/min) will be titrated for maintenance for 30 minutes, after which the subject will be allowed to recover and will be extubated as soon as appropriate.**
3) **GETA Regimen 3 (GETA 3) – each subject will be premedicated with Buprenorphine 0.01-0.02 mg/kg IM, followed by 1% propofol 2.0-7.5 mg/kg IV bolus to achieve a surgical plane of anesthesia followed by endotracheal intubation and mechanical ventilation. Propofol (10 mg/mL) will be combined with Ketamine to create a propofol-ketamine solution with a ketamine concentration of 1 mg/mL. The propofol-ketamine solution will be continuously infused at a rate of 50-300 mcg/kg/min for propofol, and a corresponding rate of 5-30 mcg/kg/min for ketamine), and will be titrated for maintenance of anesthesia for 30 minutes, after which the subject will be allowed to recover and will be extubated as soon as appropriate.**
4) **GETA Regimen 4 (GETA 4) – each subject will be premedicated with Buprenorphine 0.01-0.02 mg/kg IM , followed by alfaxalone 1-3 mg/kg IV bolus to achieve a surgical plane of anesthesia followed by endotracheal intubation and mechanical ventilation. Alfaxalone infusion will be titrated for maintenance of anesthesia (4-7 mg/kg/h) for 30 minutes, after which the subject will**

be allowed to recover and will be extubated as soon as appropriate.

Regimens for Sedation Only will include: (As described above these anesthetic regimens would be used for non-invasive procedures such as; cardiac monitoring, electrocardiography and echocardiography that generally only require sedation. These procedures will NOT be conducted on this protocol, only the sedation portion.)

1) Sedation regimen 1 (Sed 1) – each subject will receive acepromazine 0.055-0.11 mg/kg IM, SC, or IV for induction and maintenance of anesthesia for 30 minutes. Subjects will then be allowed to recover.
2) Sedation regimen 2 (Sed 2) – each subject will receive Propofol (10 mg/mL) infusion at 50-300 mcg/kg/min IV for induction and maintenance of anesthesia for 30 minutes. Subjects will then be allowed to recover.
3) Sedation regimen 3 (Sed 3) – each subject will receive a Propofol (10 mg/mL) combined with ketamine (1 mg/mL) infusion and titrated to a propofol dose of 50-300 mcg/kg/min IV, and corresponding ketamine dose of 5-30 mcg/kg/min IV), as described above, for induction and maintenance of sedation for 30 minutes. Subjects will then be allowed to recover.
4) Sedation regimen 4 (Sed 4) – each subject will receive midazolam 0.3 mg/kg IM premedication followed by alfaxalone 1-3 mg/kg IV for induction and infusion at 4-7 mg/kg/h IV for maintenance of anesthesia for 30 minutes. Subjects will then be allowed to recover.

While under anesthesia, animals will be monitored for depth of sedation/anesthesia. Close continuous monitoring allows immediate recognition and treatment of any potential compromise to body systems that may occur while under anesthesia. Cardiovascular parameters that will be monitored include heart rate, blood pressure, pulse strength and capillary refill time. Respiratory parameters include respiratory frequency, rhythm, volume and mucous membrane color. CNS parameters such as arousal and muscle tone are directly related to depth of anesthesia. Observational techniques that help indicate depth of anesthesia include the level of muscle relaxation, reflex activities, and physiologic responses to surgical stimulation. Jaw tone is one measure of muscle relaxation and if an animal attempts to close its mouth when gentle traction is placed on the mandible, more anesthesic may be needed. Purposeful movements such as swallowing or head shaking are also typically indications of too light a plane of anesthesia. The pedal withdrawal reflex (toe pinch) is commonly used to help determine the level of surgical anesthesia. Ocular reflexes can also be used to indicate anesthetic depth and include palpebral reflex (blinking that occurs when the edge of the eyelid is lightly touched), ocular position and corneal reflex. Ocular position changes with anesthetic depth. As a surgical plane of anesthesia is reached the globe rotates ventromedially (down.) With a deeper plane and continued muscle relaxation, the globe will rotate upward and return to the central position. If the globe rotates dorsomedially (up and back) the plane of anesthesia may be too deep. The corneal reflex is assessed by lightly touching the corneal surface and noting the presence of a blinking response. Physiologic responses to stimuli used to assess a level of surgical anesthesia when no purposeful movement is seen are increases in heart rate, blood pressure and respiratory rate. Increases in blood pressure or heart rate in response to stimulus may be interpreted as inadequate anesthesia and supplemental doses may be required.

These physiologic parameters and physical observations will be frequently assessed in order to identify and maintain the level of sedation or general anesthesia. For the study sedation regimens (Sed), the level of sedation will be maintained as if the subject was undergoing a non-invasive procedure that requires that the subject only remain still and calm. In addition to monitoring the above described parameters, the National Institutes of Health Modified FLACC (Face, Legs, Activity, Cry, Consolability) Scale, and Ramsay Sedation Scale (RSS) scoring systems will be used (see Fig 1 below). Subjects will be maintained at a FLACC Scale score of 0-2, and RSS score of 4-5. For general

anesthesic (GETA) regimens designed for invasive surgical procedures, subjects will be monitored as above and evaluated for responsiveness to a "surgical-like" stimulus. The surgical stimulus will be created using the toe pinch and subjects will be evaluated for physiologic response, pedal withdrawal reflex, endotracheal tube tolerance, jaw tone and ocular reflexes. Anesthetic agents will be adjusted accordingly to maintain general anesthesia.

Fig. 1

NATIONAL INSTITUTES OF HEALTH
WARREN GRANT MAGNUSON CLINICAL CENTER
PAIN INTENSITY INSTRUMENTS
Jul-03

Modified FLACC Scale							
DATE/TIME							
Face							
0 - No particular expression							
1 - Occasional grimace, withdrawn, disinterested							
2 - Frequent grimace, clenched jaw							
Legs							
0 – Normal position or relaxed							
1 – Uneasy, restless, tense							
2 – Kicking, or legs drawn up							
Activity							
0 – Lying quietly, normal position, moves easily							
1 – Squirming, shifting back and forth, tense							
2 – Arched, rigid or jerking							
Cry							
0 – No cry, whine(awake or asleep)							
1 – Moans or whimpers; occasional yipe							
2 - Crying steadily, screams, frequent yipes							
Consolability/Stress							
0 – Content, relaxed							
1 – Reassured by occasional touching, hugging or being talked to, distractible							
2 – Difficult to console or comfort							
TOTAL SCORE							

RAMSAY SEDATION SCALE							
1 – Patient anxious, agitated, restless							
2 – Patient cooperative, oriented, tranquil							
3 - Patient responds to commands only							
4 – Brisk response to light gabellar tap or auditory stimulus							
5 – Sluggish response to light gabellar tap or auditory stimulus							
6 - No response to the stimulus mentioned in items 4 and 5							

The general care of all animals under anesthesia will also include maintenance of temperature and hydration. Animals will be placed on a water-heated pad during the protocols

and body temperatures will be monitored. All animals will also have venous access through which fluids can be given to prevent dehydration. IV line placement and patency will be monitored continuously.

For each of the anesthetic (GETA and Sed) groups, all animals will be continuously monitored with pulse oximetry (SpO_2), ECG, non-invasive or arterial blood pressure, inspired oxygen concentration (FiO_2), end-tidal carbon dioxide ($EtCO_2$) and temperature. These data points as well as electrophysiologic recordings from the stellate ganglia and vagal nerve will also be collected for later analysis. Electrophysiologic recordings will be acquired from the implantable pacemaker placed under Protocol # 02243 or Protocol # 02235. A pacemaker is a medical device that can produce heart beats on command and can also track cardiac electrical activity. Similarly, an implanted radio telemetry device, also placed per Protocol # 02243 or Protocol # 02235, will also continuously record the cardiac rhythm and nerve activity in response to premature heart beats and different anesthetic medications. Additional parameters to be collected and evaluated will include mean heart rate (R to R interval), corrected QT interval (QTc) and ventricular effective refractory period (VERP). Nerve recordings from the stellate ganglion and vagus nerve will also be analyzed for signal quality (amplitude, latency, etc) variance between the different anesthetic regimens and baseline measurements. Additional hemodynamic measurements (if available from indwelling arterial and/or venous catheters from Protocol # 02243 or Protocol # 02235) including systolic/mean/diastolic arterial blood pressure and pulse pressure will also be assessed and analyzed as compared to baseline measurements. Data will be recorded for 5 minutes prior to the induction of anesthesia in order to establish baseline values, and will continue to be collected for 30 minutes after the end of the anesthetic exposure during the immediate recovery period. The animals will be given a minimum of 2 days to recover between anesthetic exposures and to minimize the effect of the agents on subsequent anesthetics.

Postoperative care falls into 3 phases: recovery from anesthesia, the immediate postoperative period and the long-term postoperative period. During the 1st phase the major concerns are controlling the animal's airway, assisting postural changes and preventing heat loss. For subjects recovering from general anesthesia, the endotracheal tube will be maintained to prevent aspiration of gastric contents and will not be removed until a swallowing reflex is seen. During the second phase, animals that can't maintain sternal recumbancy will be turned from side to side frequently (every 15 minutes) to prevent blood pooling in the dependent lung. Supplemental heat will also be provided via warm water-heating pads or blankets, and subjects will be watched closely to prevent overheating. Animals will also be frequently evaluated for mucous membrane color, respiratory rate and pattern, return of reflexes after anesthesia, jaw tone, heart rate and rhythm and body temperature.
The long term postoperative period will extend a minimum of 2 days in order to allow for adequate recovery return of baseline level of function and clearance of anesthetic medications (based on estimated elimination half-life). The times the animal is sternal, standing, eating, drinking and producing urine will be documented on the surgical form. We will require at least 36 hours of normal behavior as observed prior to the anesthesia (normal activity, normal eating/drinking, normal stool/urine output) before continuing with the next regime. This would assume it should take less than 12 hours to fully recover from all anesthesia regimens. Should the animal show any signs of nausea (drooling/vomiting/anorexia), anti-emetics (Famotidine 0.5-1 mg/kg PO or IV or Metoclopramide 0.2-0.5 mg/kg IV) will be started to treat the symptoms. If these symptoms last longer than 48 hours, the animal will be removed from the study and not be given any further anesthetics on this protocol. Additionally, the canine will be weighed at least twice weekly while on this protocol and if the weight changes by 5%, daily weights will be taken. If weight drops by 10% or more, the animal will be removed from the protocol.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

►
The sample size was estimated based on a predicted 10% decrement in nerve signal amplitude from base line, with a corresponding SD. A sample size of 8 test subjects will be needed to achieve an alpha of 0.05 and a power of 0.8. .

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► Administering Anesthesia

In order to evaluate the effects of anesthetic agents on cardiac conduction and hemodynamic stability, the following anesthetic regimens will be utilized. All animals undergoing a procedure requiring anesthesia will be monitored with continuous pulse oximetry (SpO_2), ECG, non-invasive or arterial blood pressure, inspired oxygen concentration (FiO_2), end-tidal carbon dioxide ($EtCO_2$) and temperature. IV line placement and patency will be monitored continuously during these procedures. Animals will be monitored for consciousness by toe pinching and looking for blink reflex. Distress will be evaluated by closely and continuously monitoring and recording heart rate and blood pressure.

1) Brevital and Isoflurane

The animal will be administered Brevital 6-10 mg/kg IV bolus, via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Inhaled 1-4% isoflurane will be titrated for anesthetic maintenance for 30 minutes and then allowed to recover.

2) Propofol infusion

The animal will be administered 1% propofol 2.0-7.5 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Propofol infusion (10 mg/mL at 50-300 mcg/kg/min) will be titrated for maintenance for 30 minutes and then allowed to recover.

3) Propofol and Ketamine

The animal will be administered 1% propofol 2.0-7.5 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Propofol infusion (10 mg/mL at 50-300 mcg/kg/min) combined with Ketamine infusion (1 mg/mL at 5-30 mcg/kg/min) will be titrated for maintenance for 30 minutes and then allowed to recover.

4) Alfaxalone

The animal will be administered alfaxalone 1-3 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Alfaxalone infusion will be titrated for maintenance (4-7 mg/kg/h) for 30 minutes and then allowed to recover.

Data Collection and Statistical Analysis:

Both non-invasive hemodynamic data, and electrophysiologic, neurophysiologic/and hemodynamic data from the monitoring devices previously placed in protocol # 02243 or 02235 will be collected and will be used to evaluate the impact of anesthetic agents on cardiovascular function.

Statistical analysis will be performed using with SAS/STAT® Software (SAS Institute, Inc. Cary, NC). Data will be statistically analyzed in comparison to baseline measurements and across groups. Paired-t- test will be used to compare baseline versus final parametric variables, and one-way ANOVA (with Tukey posthoc analyses) will be used for multiple group comparisons.

All data will be reported as the mean +/- SD and a P value less than 0.05 will be considered statistically significant.

Results of this study will be utilized to help identify the anesthetic regimen with the least impact on the cardiovascular system and allow the acquisition and capture of optimal electrophysiologic signals.

D. **Species.** Justify the choice of species for this protocol.

►
The canine model has been chosen for this study because of the preexisting protocols (Protocol # 02243 or Protocol # 02235) that aim to study cardiovascular disease and related electrophysiology. Canine models share a similar electrical system to humans and therefore any electrophysiological changes in anesthesia could be related to humans.

Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. PI

Name [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Administering Anesthesia	[redacted] experience where he provided anesthesia for rat surgical procedures.

2. Other research personnel (copy the lines below for each individual)

Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Administering Anesthesia	[redacted]

2. Other research personnel (copy the lines below for each individual)

Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Administering Anesthesia	[redacted]

Name► [redacted]
Animal research experience ► [redacted]

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Administering Anesthesia	[redacted]

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)

Name►

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted]	[redacted]	[redacted]	**no**
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	**no**
[redacted]	[redacted]	[redacted]	**no**

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"
► Dr. Rice is a board certified anesthesiologist and therefore is the most qualified of the personnel to administered anesthesia. He will provide any additional training and guidance that is needed.

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

Name	Enrollment in OHSP		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
[redacted]	(X)			**yes**
[redacted]	(X)			**yes**
[redacted]	(X)			**yes**
[redacted]	(X)			**Yes**

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

► ( ) Yes. Describe them ►

► (X) No.

Animals Requested

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Canines, mongrel	**M/F**	**20-30 kg**	[redacted]	**Conditioned**

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures►						
Species / Experimental Group / Procedures(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category B TOTAL**

USDA Category C

Procedures►Induction of anesthesia						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**
	8	**0**	**0**	**0**		**8**

USDA Category D

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**

USDA Category E

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**

Canine						

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
Canine	**8**					**8**

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► (X) This protocol does NOT include any Category D procedures.

► () This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

Procedure	Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for the monitoring	Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered)

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► (X) This protocol does NOT include any Category E procedures

► () This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.

►

Veterinary Care and Husbandry

L. **Veterinary Support**.

1. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

Name► [redacted]
email contact► [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted► [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ►[redacted]

M. **Husbandry.** As a reference <u>for the animal husbandry staff</u>, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

a. Species	b. Type of housing*	c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
Canines	**Standard, see below**	**1**	**yes**	**17**

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
► **Standard – raised floor, chain link, 3x6 feet minimum**

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
► **Dogs are housed singly in chain link runs, but can socialize as each room has at least 2 housing units that have no solid divider. Dogs are able to see and smell one another.**

***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

a. Species	b. Description of Enrichment*	c. Frequency
Canine	**Standard, see below**	**daily**

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here.

► **Daily milk bones, nylon chew bones rotated weekly**

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.
►

► ( ) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.
►

► ( ) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
►

► (**X**) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed.

► (**X**) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]	[redacted]	(X)	( )

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table.

Name of Non-VA Facility	Is this facility accredited by AAALAC?		Building	Room Number
	Yes -- enter status*	No**		
	( )	( )**		

	( )	( )**		
	( )	( )**		

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".

► (**X**) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (**X**) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

Procedure	Surgical?		Bldg/Room Number	Requires transport through non-research areas?	
	Yes	No		Yes – describe method of discreet transport	No
General and sedation anesthetic protocols		**(X)**	[redacted]	**( )**	**(X)**

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

Body Fluid, Tissue, or Device to be	Collected AFTER	Collected BEFORE Euthanasia

Collected	Euthanasia	Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
N/A	**( )**	**( )**	**( )**	**( )**
	( )	**( )**	**( )**	**( )**

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► () Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► (X) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► Protocol # 02243 or Protocol # 02235All animals will be closely and continuously monitored for pain and distress. Oxygen saturation, blood pressure, heart rate, capillary refill time and temperature will be recorded and the anesthesic agent will be adjusted according to normal parameters during surgery. Acceptable heart rate parameters while the animal is under anesthesia are 40-130 bpm. Acceptable blood pressure parameters during surgery are systolic 70-110 mmHg, diastolic 40-60 mmHg .Blood pressure and heart rate is carefully monitored and managed within this range and efforts will be made if the blood pressure or heart rates changes during the procedure, However, **in the event that the heart rate drops below 40 bpm or increases above 130 bpm, or the systolic blood pressure falls below 70, every effort will be made in order to resuscitate and stabilize the animal. Basic and advanced cardiac life support interventions may include giving additional IV fluids and/or ionotropic (such as epinephrine) or vasopressor medications (such as phenylephrine or vasopressin), chest compressions, and external pacing/defibrillating. These interventions will be continued until the animal recovers or efforts are deemed to be futile, at which point the animal will be allowed to expire via anesthetic overdose.**

U. **Termination or removal from the protocol**. Complete each of the following that applies:

► (X) Animals will NOT be euthanatized on this protocol. .
► The animals will be returned to protocol Protocol # 02243 or Protocol # 02235 for completion of those approved procedures. Euthanasia due to reaction to the anesthetic as described in Section T is not part of the planned study.

► () Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
( )	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►		( )	( )	( )
( )	Anesthetic overdose Agent► Dose► Route of administration►		( )	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Other (Describe) ►		( )	( )	( )

1. For each of the methods above that is designated as “Conditionally Acceptable” by the AVMA, describe how the conditions for acceptability will be met:
   ►

2. For each of the methods above that is designated as “Unacceptable” by the AVMA, give the scientific

reason(s) that justify this deviation from the AVMA Guidelines:
►

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.
►

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
   ► **Carcass should be refrigerated and saved for autopsy. Staff should be contacted immediately.**

   b. Describe how the PI's staff should be contacted.
   ► (**X**) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
   Name► [redacted]
   Contact Information► [redacted]

   ► ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
   ►

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
		Items:	(X)**
		Items:	(X)**

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ► **The animal will be briefly restrained while inserting an IV catheter for anesthesia induction. The pain felt during this procedure will be a quick pinch feeling as the catheter is inserted into either the left of right cephalic veins. Animals may also experience a mild degree of distress and/or disorientation upon recovering from anesthesia.**

Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
PubMed	**1/24/2017**	**1970 - 2017**	Anesthesia administration	**Propofol, ketamine, alfaxalone, isoflurane, canine anesthesia, cardiovascular, hemodynamic, electrophysiology**	**(X)**	**(X)**	**(X)**	**(X)**
ALTWEB	1/24/201	1970-2017	Anesthesia	**Propofol,**	(X)	(X)	(X)	(X)

	7		administration	**ketamine, alfaxalone, isoflurane, canine anesthesia, cardiovascular, hemodynamic, electrophysiology**				

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
   ► **The purpose of this study is to identify the optimal anesthetics for use in canine cardiovascular procedures and/or electrophysiological studies and therefore cannot be completed in another species or by computer-based modeling.**

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
   ► **By sharing the animals with the ACORP protocols (Protocol # 02243 or Protocol # 02235), we have dramatically reduced the number of animals needed. No additional animals will used for our study.**

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.

   ► **Our project is designed to minimize pain and distress, as well as use as few animals as possible. We will follow AWA recommendations to minimize distress and pain. By finding the appropriate anesthetic regimen for these studies, accurate electrophysiological data can be acquired without comprising the comfort level of the animal.**

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
   ► **After thorough literature search it was determined that no other study exists which has specifically addressed our scientific inquiry.**

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

   a. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Phar-macy	Non-VA

Brevital	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Ketamine	(X)	( )*		(X)	( )	(X)	( )
Isoflurane	(X)	( )*		(X)	( )	(X)	( )
Propofol	(X)	( )*		(X)	( )	(X)	( )
Alfaxalone	(X)	( )*		(X)	( )	(X)	( )

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.
►

b. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

► (X) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.

► ( ) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

► ( ) Other. Explain►

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

► (**X**) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

► (**X**) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (**X**) Appendix 3, "Biosafety"
► ( ) Appendix 4, "Ante-mortem Specimen Collection"
►) Appendix 5, "Surgery"
► ( ) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

Item	SOP		Approval Date
	Title	ID	
C.2.c			
M.1			
M.2			
U.4.a			
U.4.b			
V			

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

a. **Certification by Principal Investigator(s):**

I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue

without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

I understand that further IACUC approval must be secured before any of the following may be implemented:

- Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
- Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
- Performing any additional procedures not already described in this ACORP;
- Use of any of these animals on other protocols, or by other investigators.

I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Name(s) of Principal Investigator(s)	Signature	Date
[redacted]	[redacted]	[redacted]

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

  ► ( ) No minority opinions were submitted by any IACUC participant for inclusion.

► ( ) Minority opinions submitted by IACUC participants are copied here
►

► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages►)

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of IACUC Chair	Signature	Date

2. **Appendix 2. Antibody Production.** No signatures required.

3. **Appendix 3. Biosafety.**

a. **Certification by PI(s) and IACUC Officials:**

We certify that:

- Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

- All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]	[redacted]	[redacted]
Name of Institutional Veterinarian	Signature	Date
Name of IACUC Chair	Signature	Date

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";
- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee	Signature	Date

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee	Signature	Date

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:
   - To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
   - The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
   - The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
   - The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
   - Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:

     - Identification of each animal such that care for individual animals can be documented.
     - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
     - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
     - Daily records covering at least the period defined as “post-operative” by local policy.
     - The signature or initials of the person making each entry.

.

Name(s) of Principal Investigator(s)	Signature(s)	Date

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

   a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

Name(s) of Principal Investigator(s)	Signature(s)	Date

b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of the Manager of the Human Patient Care Equipment	Signature	Date

c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of Chief of Staff	Signature	Date

Name of Director or CEO of the Facility (Hospital or Clinic)	Signature	Date

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

a. **Certification by the Principal Investigator(s).**

I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

I further certify that:

- Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
- All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
- Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
- Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

Name(s) of Principal Investigator(s)	Signature(s)	Date

b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date

Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of VISN Regional Safety Officer	Signature	Date

9. **Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

ACORP APPENDIX 3
BIOSAFETY
VERSION 4

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

Material (Identify the specific agent, device, strain, construct, isotope, etc.)	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**						
		Toxic Agent (Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent
Isoflurane	**Richmond VA pharmacy**						**(X)**	
Brevital	**Richmond VA pharmacy**						**(X)**	
Buprenorphine	**Richmond VA pharmacy**						**(X)**	
Ketamine	**Richmond VA pharmacy**						**(X)**	
Propofol	**Richmond VA pharmacy**						**(X)**	
Alfaxalone	**Richmond VA pharmacy**						**(X)**	
Acepromazine	**Butler Schein**						**(X)**	
Epinephrine	**Richmond VA pharmacy**						**(X)**	
Norepinephrine	**Richmond VA pharmacy**						**(X)**	
Vasopressin	**Richmond VA pharmacy**						**(X)**	

Midazolam	**Richmond VA pharmacy**						**(X)**	
Metoclopramide	**Richmond VA Pharmacy**						**X**	
Famotidine	**Richmond VA Pharmacy**						**X**	

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in Item 1 above:

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose (e.g., mg/kg, CFU, PFU, number of cells, mCi) and Volume (ml)**	**Diluent* or Vehicle***	**Route of admin**	**Frequency or duration of admin**	**Reason for Administration and Expected Effects**	**Location of Further Details in this ACORP (specify "Main Body" or "App #" and identify the Item)**	**Administration Under Anesthesia, sedation, or tranquilization (Y/N)**
Brevital	**6-10 mg/kg to effect**	**Normal saline**	**IV**	**Once for for each animal**	**Induction of anesthesia**		**Y**
Isoflurane	**1-4%**	**None**	**Inhalation**	**Once for eavch animal - Continuous for 30 min**	**Maintenance of anesthesia**		**Y**
`Buprenorphine	**0.01-0.02 mg/kg**	**None**	**IM**	**Once for each general anesthetic regimen**	**Sedation/pain control**		**Y**
Ketamine	**2-4mg/kg to effect, and/or 5-30 mcg/kg/min IV**	**None**	**IV**	**Will be used 4x per animal – each time for 30 min**	**Sedation**		**Y**

Alfaxalone	**1-3 mg/kg IV for induction and infusion at 4-7 mg/kg/h IV for maintenance**	**None**	**IV**	**Will be used 4x per animal – each time for 30 min. 1-3 mg/kg IV for induction and infusion at 4-7 mg/kg/h IV for maintenance**	**Sedation**		**Y**
Propofol	**2.0-7.5 mg/kg IV for induction, or infusion at 50-300 mcg/kg/min**	**None**	**IV**	**Will be used 4x per animal – each time for 30 min. 2.0-7.5 mg/kg IV for induction, or infusion at 50-300 mcg/kg/min**	**General anesthesia or Sedation**		**Y**
Acepromazine	**0.055-0.11 mg/kg**	**None**	**IM, SC, or IV**	**Will be used once per animal for sedation - 0.055-0.11 mg/kg**	**Sedation**		**Y**
Epinephrine	**0.1 – 10 mcg/kg**	**None**	**IM, IV, ETT**	**As needed for resuscitation**	**Hemodynamic Instability**		**Y**
Norepinephrine	**0.1 – 10 mcg/kg**	**None**	**IV**	**As needed for resuscitation**	**Hemodynamic Instability**		**Y**
Vasopressin	**0.1 – 10 Units**	**None**	**IV**	**As needed for resuscitation**	**Hemodynamic Instability**		**Y**
Midazolam	**0.3 mg/kg**	**None**	**IV, IM**	**Will be used once per animal for sedation**	**Sedation**		**Y**
Metoclopramide	**0.2-0.5 mg/kg**	**None**	**IV**	**Up to twice a day as needed**	**antiemetic**		**N**
Famotidine	**0.5-1 mg/kg**	**None**	**PO or IV**	**Up to twice a day as needed**	**Antiemetic**		**N**

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)
All diluents are pharmaceutical grade.

3. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

1) Brevital and Isoflurane

The animal will be administered Brevital 6-10 mg/kg IV bolus via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Inhaled 1-4% isoflurane will be titrated for anesthetic maintenance for 30 minutes, and then the animal will be allowed to recover.,

2) Propofol infusion

The animal will be administered 1% propofol 2.0-7.5 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Propofol infusion (10 mg/mL at 50-300 mcg/kg/min) will be titrated for maintenance for 30 minutes, and then the animal will be allowed to recover...

3) Propofol and Ketamine

The animal will be administered 1% propofol 2.0-7.5 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Propofol infusion (10 mg/mL at 50-300 mcg/kg/min) combined with Ketamine infusion (1 mg/mL at 5-30 mcg/kg/min) will be titrated for maintenance for 30 minutes, and then the animal will be allowed to recover. ..

4) Alfaxalone

The animal will be administered alfaxalone 1-3 mg/kg via either the left or right cephalic veins until the animal is unconscious, followed by endotracheal intubation and mechanical ventilation. Alfaxalone infusion will be titrated for maintenance (4-7 mg/kg/h) for 30 minutes, and then the animal will be allowed to recover. . ..

Metoclopramide and Famotidine are given with anesthesia either orally or IV and no anesthesia is required..

a. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
►

4. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

Name of Toxic Agent	Mutagenic	Teratogenic	d. Select Agent?	e. Other specify toxic properties

				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

5. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name and BSL Number of Infectious Agent	a. ABSL Number *	b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?		
			Not a Select Agent	Select Agent used in Sub-threshold quantities	Select Agent that Requires Registration/Approval

N/A		(Yes/No)	( )	( )	( )**

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ►
Justification for applying ABSL measures that are less protective than those recommended ►

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

6. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Biological Agent	Screening for Infectious Agents
N/A	

7. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Radioactive Agent (specify the isotope)	Authorized Individual	Approving Committee or Official
N/A		

8. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

Name of Agent that Contains Recombinant Nucleic Acid	Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt
N/A	( )	( )
	( )	( )

9. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3

Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress
N/A		

10. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

    a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk
N/A			

    b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.
    ►

11. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP.

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Richmond, VA - 652	None	A Comparison of Canine Anesthetic Regimens to Optimize Hemodynamic Stability and Quality of Electrophysiologic and Neurophysiologic Data Acquisition

ACTION NEEDED BY IACUC
The IACUC must review the concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s) and the revised ACORP(s) must be forwarded to the CVMO for archiving.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted] or [redacted].

REVIEWER FEEDBACK

ACORP Item number(s)	Comments/Concerns
ACORP (dog)	The primary goal of the proposed study is to identify the anesthetic regimen that maintains cardiac function and optimizes the collection of neuro-/electro-physiological measurement data. Dogs, which have been implanted with pacemaker and neuro-/electro-physiologic recording devices (IACUC approved protocols # 02243 and/or # 02235), will be transferred to this protocol once they have met well-defined inclusion criteria. The animals will be anesthetized with various anesthetic agents or combinations of anesthetics agents to determine the optimal anesthesia regimen. This protocol only involves anesthetic procedures; surgery will not be performed. These dogs will be transferred back to protocols # 02243 and/or # 02235 once the study is complete. The principal investigator is a board certified cardiothoracic anesthesiologist and the other members of the research team are experienced in canine thoracic surgery and anesthesia. The investigator is commended for the comprehensive anesthesia monitoring and post procedural care plans. A few aspects of protocol should be clarified. An appendix to this review provides additional information for the IACUC's consideration. The specific numbered comments provided below must be reviewed by the IACUC, to determine what responses are needed. These actions must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP and the revised ACORP provided to the CVMO for archiving.
Item C.2	Dogs will be randomly assigned and undergo each of the eight anesthetic regimens (four general anesthesia protocols and four sedation protocols) for 30 minutes. "A minimum of 2 days will separate each anesthetic exposure in order to allow for adequate recovery return of baseline level of function and clearance of anesthetic

(cont.)

	medications (based on estimated elimination half-life)." Although, the overall experimental plan is clear; it would be helpful to: (1) Address the timeline of the study and explain the order in which an individual dog(s) will undergo the various anesthesia protocols. (2) The investigator indicates that 8 dogs are needed in item C.2.b but a more clear explanation of the accounting would be helpful.
Items D and W	The investigator has explained that dogs are the chosen species for this study because these dogs are prepared for this study by first undergoing the procedures performed under protocols # 02243 and# 02235. Nonetheless, it would be helpful to briefly discuss why dogs are the preferred model as opposed to other cardiac models.

Appendix - Additional Suggestions for Improvement

Comment 1: <u>Part B.</u> This section would be strengthened for the lay reader by starting it with the relevance of this work to human heart disease. Also, the specific relevance to Veterans' health is not specified. Try putting something like this at the beginning of the section:

The focus of our research is heart disease, specifically cardiomyopathy which the CDC website says affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 10/10/17).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 10/10/17).* We have found that the best way to study cardiomyopathy is with dogs, and this project will determine the best anesthetics to use for this work. It may also lead to better anesthetic choices for veterinarians doing dog surgeries.

Comment 2: <u>Part D:</u> The justification for using dogs could be made stronger to the lay reader. Try something like this:

Our group uses canines because their physiology is very similar to humans, including having a His-Purkinje system located in the endocardium which pigs and other larger animals do not have. Unfortunately the electronic pacemakers, leads, and radiotelemetry devices available for this kind of research are too large for small species such as rabbits, rats, or mice. This study will investigate the impact of several different anesthetic regimens on dogs from pre-approved protocols (Protocol # 02243 and Protocol # 02235) and no new animals will be needed for this study.

(cont.)

Comment 3: **Part H:** **In order to avoid confusion on the part of the reader, it would be helpful to add a note underneath table H like this:**

Please note: This study will use dogs from pre-approved protocols (Protocol # 02243 and Protocol # 02235) and no new animals will be purchased for this study.

Comment 4 Part W1 table: The searches in the original table will only bring up individual papers that include all of the search terms listed, which may eliminate important papers. Running these searchers as written in the original table yields no papers at all. See below for a suggested approach to literature searches.

1. Document the database searches conducted.

   List each of the potentially painful or distressing procedures included in this protocol.

   ► (X) **N/A The animal will be briefly restrained while inserting an IV catheter for anesthesia induction. The pain felt during this procedure will be a quick pinch feeling as the catheter is inserted into either the left of right cephalic veins. Animals may also experience a mild degree of distress and/or disorientation upon recovering from anesthesia.**

   ► ( ) **Painful or distressing procedures:**

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
ALTBIB (Search for Citations with Animal Use Alternatives as the main topic)	**12-21-2017**	**2000-2017**	**N/A – this search was for alternatives to using animals.**	**anesthesia, cardiovascular**	X	X	X	
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-21-2017**	**2000-2017**	**Inserting a catheter into the cephalic vein**	**cephalic vein catheter**	X	X	X	
PubMed	**12-19-2017**	**1966-2017**	**N/A**	**anesthesia AND cardiovascular electrophysiology AND**				X

(cont.)

				canine AND (propofol OR ketamine OR alfaxalone OR isoflurane)				

Comment 5: <u>part W2 (replacement)</u> This section would be strengthened by adding sentences like these to go with the searches in section W1 above:

We ran a search on ALTBIB specifically for alternatives to using animals at all for this work, and got only three papers. One of the papers was a review article and the other two were actually animal studies. There are no computer models or in vitro models available for this work.

Comment 7: <u>part W4 (refinement).</u> The discussion of refinements in this section is quite good. It would be strengthened by adding a comment on the search for refinement in the table above. Try something like this:

We ran a search on ALTBIB for "cephalic vein catheter" and got only one paper. This paper did not have a refinement over our present method for inserting the cephalic vein catheter.

Comment 8: <u>part W5 (lack of unnecessary duplication)</u> As noted above, the searches in the original table will only bring up papers that include <u>all</u> of the search terms listed, which may eliminate many important papers. Try something like this:

Our study will focus on determining the best anesthetic drug combination for studies on cardiac electrophysiology and related functions in dogs so the data will be as relevant to human patients as possible. We ran a PubMed search for "anesthesia AND cardiovascular electrophysiology AND canine AND (propofol OR ketamine OR alfaxalone OR isoflurane)" and got only 7 papers. One was actually on swine. The other papers did not include alfaxalone or ketamine, two of the drugs we are studying. To properly compare the different anesthetic drug combinations we are studying and determine the best one, they must be run in parallel with the same procedures and equipment. This has not been done before.

(cont.)

Literature search 652 Richmond [redacted]

[redacted] How is this research relevant to Veterans health?

The focus of our research is heart disease, specifically cardiomyopathy which the CDC website says affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 3/10/18).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 3/10/18).* We have found that the best way to study cardiomyopathy is with dogs, and this project will determine the best anesthetics to use for this work. It may also lead to better anesthetic choices for veterinarians doing surgeries on dogs.

This project only studies dogs while they are anesthetized for other projects – *no additional dogs are used for this study.*

2) Is this work unnecessarily duplicating work already documented in the literature?

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
PubMed	3/11/18	All available years	anesthesia AND cardiovascular electrophysiology AND canine AND (propofol OR ketamine OR alfaxalone OR isoflurane)	7

Our study will focus on determining the best anesthetic drug combination for studies on cardiac electrophysiology and related functions in dogs so the data will be as relevant to human patients as possible. We ran a PubMed search for “anesthesia AND cardiovascular electrophysiology AND canine AND (propofol OR ketamine OR alfaxalone OR isoflurane)” and got only 7 papers. One was actually on swine. The other papers did not include alfaxalone or ketamine, two of the drugs we are studying. To properly compare the different anesthetic drug combinations we are studying and determine the best one, they must be run in parallel with the same procedures and equipment. This has not been done before.

The work requires collecting accurate data on cardiac function and electrophysiology, and it is well established that anesthetic agents can depress cardiac function and/or impair electrophysiological and neurophysiologic monitoring. The goal of this study is to determine which anesthetic regimen permits the most accurate cardiac data collection in dogs so the data will be as relevant to human patients as possible.

3) Could this work be done in computer models or in vitro (tissue culture)?

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
ALTBIB Citations with Animal Use Alternatives as the main topic	3/10/18	All available years	anesthesia AND cardiovascular electrophysiology	0

An ALTBIB search for “alternatives to animal use” brought up no papers. No compuer or in vitro models were found.

4) Could it be done in non-mammals or in other mammals?

In principle this work could be done on other species, however these dogs are already being used for cardiac physiology research.

Dogs are the species of choice for this kind of research for two reasons: 1) Atrial fibrillation can be readily induced in dogs and 2) The group has been using dogs for over 25 years. In order for the new data to be comparable with the previously collected data, they need to continue to use dogs. Switching to another species would to some degree be starting over, and require many more animals than this study will use.

5) **Are the methods used the best available (least painful or distressing to the dogs)?**

The dogs will be fully anesthetized during these procedures, so they will feel no pain or distress.