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►**Richmond 652**

3. Protocol Title► Effect **of chronic premature ventricular contractions on the remodeled ischemic heart.**

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

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): pending

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►

      (2) 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.
►

Cardiovascular disease is the number one killer in America today. Over the last few years advances in Interventional Cardiology have reduced the number of patients that die suddenly from a heart attack (myocardial infarction). However, in some patients we are unable to completely open blocked blood vessels of the heart, or even get to them in time to prevent an infarct. In addition, many patients, especially women and diabetic patients can have silent infarction that is left untreated. In the patients that survive the initial insult of myocardial infarction, there is the problem of left ventricular (LV) remodeling. Left ventricular remodeling is the combination of cellular, and extracellular fibrotic changes that leads to worsening heart function, and ultimately will result in a condition known as ischemic (restriction in blood supply) cardiomyopathy. In this condition, the heart becomes weaker, as the remaining uninjured heart muscle (myocardium) fails to compensate for the dead myocardium that results from the initial heart attack. Our knowledge of the mechanisms that drive LV myocardial remodeling are limited, and therefore considerable effort has been given to better understanding how the remodeling process results in worsening myocardial dysfunction. To date no studies have looked at determining how arrhythmia (abnormal electrical activity in the heart) and ventricular remodeling are related. Most researchers would likely state that the ventricular ectopy (electrical activity originating from the lower chambers of the heart; i.e., Premature Ventricular Contractions (PVC) or Ventricular Tachycardia (VT) is a result of diseased myocardium, destabilized by the remodeling process. However, more recent work with PVC induced cardiomyopathy, including research from our lab, has shown that the ventricular ectopy is capable of inducing adverse LV remodeling in an otherwise normal heart. This study is relevant because it seeks to determine the effects of PVC induced myocardial dysfunction in the setting of previously established ischemic cardiomyopathy (heart failure resulting from prior myocardial infarction). Ischemic cardiomyopathy is the etiology of more than half of all patients with clinical heart failure. Furthermore, the presence of scar in the myocardium makes these patients more susceptible to ventricular ectopy such as PVCs or VT. These patients will have a poor prognosis compared to patients with heart failure from another cause. This will be the first model to investigate the role of excessive PVCs in ischemic cardiomyopathy, and will enable us to better understand how increased burden of ventricular ectopy effects LV remodeling in this population. Furthermore, the comparison of this data to our prior work of PVC induced cardiomyopathy in otherwise normal hearts should give us some insight into why patients with ischemic cardiomyopathy have a worse prognosis. If we

find a significant increase in LV remodeling from PVCs induced cardiomyopathy in ischemic hearts it may lead to more aggressive treatment of PVCs in this population.

In this project we aim to determine how increased PVC burden effects remodeling in a canine model of chronic ischemic cardiomyopathy. Dogs are historically used in research investigating cardiac arrhythmia because of their proven similarities to human cardiac conduction. These experiments would be impossible in smaller animals because of their faster heart rates and the inability to implant the relatively large monitoring devices that will be required to complete these experiments. The animals involved in this study will be anesthetized for surgery, and further data collection should not cause any perceivable pain. All animals will be humanely euthanized at the end of the study.

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.
►

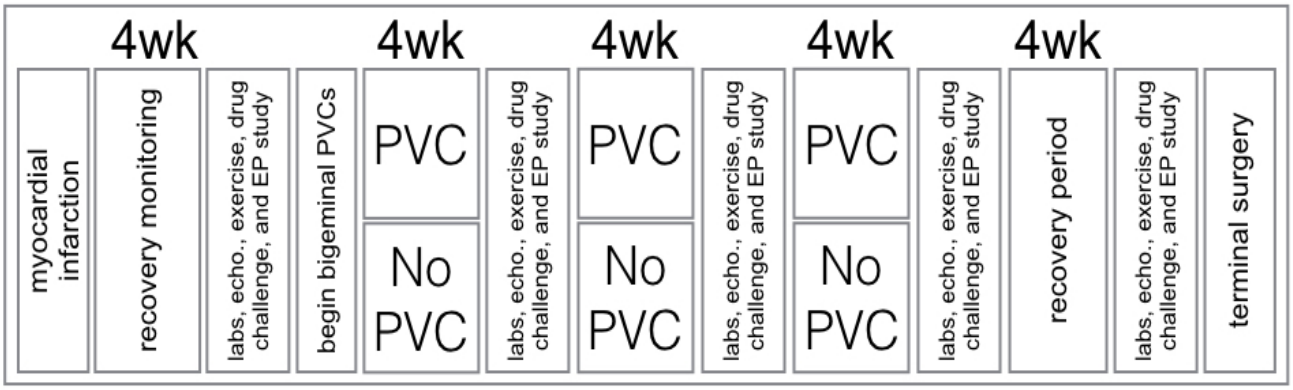
This study will determine how premature ventricular contractions (PVCs) contribute to myocardial dysfunction, and disease in a model of ischemic cardiomyopathy which is heart failure caused by heart attacks. We will use a dog model of heart attack, also known as myocardial infarction, because dogs are well suited for the subsequent studies of arrhythmia. Our lab also has extensive experience with the dog model for PVCs and myocardial infarction. All dogs in this study will be anesthetized and prepped for surgery, and then undergo electrocardiogram, and blood draw in the OR prior to operation to establish baseline cardiac function, and baseline enzyme levels. All animals will then undergo an initial left sided thoracotomy with visualization of the left anterior descending or left circumflex artery. While the chest is open, we will implant all animals with a pacemaker that is programmed to deliver PVCs to the epicardial surface of the right ventricle. We will then implant our Data Science International (DSI) telemetry device for direct autonomic, and epicardial electrocardiographic (recording the electrical activity of the heart) (ECG) recording in these animals. A telemetry device communicates measurements and other data which can be collected and transmitted. At this point all of the animals will undergo ligation of the selected artery with subsequent injection of 0.2-1.0 mls liquid latex into the coronary artery to produce an area of occlusion and subsequent infarct from the resulting lack of blood flow. We will monitor the animal for 20 minutes post infarction, create extrathoracic pockets for device implantation, and close the chest. Animals will be monitored in a recovery cage until they are moving (sternal). On the night after surgery, we will begin recording endogenous ECGs with concurrent autonomic activity and blood pressure. The telemetry will be subsequently collected every 14 days for 24-72 hours. After 4 weeks of recovery canines will undergo repeat transthoracic echocardiography to determine the degree of initial myocardial infarction induced dysfunction in these hearts. This will be followed by exercise treadmill testing to establish baseline function, and symptoms, followed by pharmacologically induced tachycardia (fast heart rate), and ventricular programmed stimulation to test for arrhythmia inducibility as described below. The animals will then be returned to their cages, and the PVC generating pacemaker will be activated for half of the infarcted animals. All animals will be followed for an additional 12 weeks with biweekly 24-72 hour telemetry and monthly treadmill tests, pharmacologic tests, PVC challenges and ventricular programmed stimulation to determine how LV dysfunction is progressing in the PVC group compared to the non-PVC group. At the end of this period the pacemakers generating PVCs will be deactivated, and all animals will then be subjected to echocardiograms, treadmill tests, pharmacologic tests, PVC challenges, and ventricular programmed stimulation to determine the final degree of cardiac dysfunction, symptoms, and inducibility of arrhythmias. Some of the animals will be allowed to recover function off of PVCs for an additional 4 weeks with repeated studies as above. At the end of these experiments all animals will undergo a final terminal surgery with subsequent harvesting of tissue for molecular and microscopic analysis of the diseased hearts

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. ►

This experiment will be based on establishing a reproducible model of post myocardial infarction remodeling in all animals. All 20 animals will undergo the initial surgery to create a full thickness myocardial infarction of the left ventricle by injecting liquid latex. Prior to the infarction, they will all receive baseline echocardiograms (ultrasound imaging of the heart) to establish baseline function. Immediately before myocardial infarction they will receive a DSI telemetry device implant, and a pacemaker/ defibrillator device implant. All animals will then be monitored for 4-6 hours in the lab for recovery before returning to the cage overnight. They will have active DSI monitoring throughout the recovery period and overnight. All animals will have labs drawn immediately after the infarction, and again at the end of recovery. We are most concerned for arrhythmias during the initial period so we will continue a close monitoring schedule with hourly checks by staff during business hours (8-5) for the first two days post OP or longer if problems are noted, lab draws daily or every 12 hours, and blood pressure checks at least every 12 hours for the first two days postop. In addition, internal monitoring will be continued for 4 weeks using the implanted pacemaker/defibrillator. If abnormal heart rate is noted during business hours, animal will be closely monitored for at least 2 hours after the event. The pacemaker/defibrillator will be programed to give accelerated tachycardia pacing (ATP) to treat any ventricular tachycardia. That is any ventricularly originating heart rate greater than 160 beats per minutes that is sustained for 30 beats or more. After four weeks all animals will have echocardiograms to establish the area of reduced or absent wall contraction corresponding to the region of dead (infarcted) myocardium. Animals with significant myocardial injury will be randomized to continuous PVCs or continued monitoring for the next three months. During this period, initial and monthly repeat echocardiograms will be obtained along with exercise treadmill tests, EP studies (programmed pacemaker stimulation to induce arrhythmias), holter monitoring and basic labs. At the end of the 12-week period PVCs will be discontinued and animals will be monitored for an additional 4 weeks to determine how much cardiac function is recovered. Canines will have a final echocardiogram, EP study, pharmacologic testing, and treadmill test then they will be euthanized and the hearts will be harvested for morphometric analysis, histologic examination and further molecular studies.

No more than two animals will undergo survival surgeries within a 7 day period (Sunday to Saturday). At least two research personnel will be available to provide supportive treatment over weekends and holidays through one month post-surgical date. After one month, with no complications for at least 2 weeks, at least one research personnel will be available on weekends and holidays to provide any supportive care, if needed.



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

This aim is based on our ability to create a reproducible myocardial infarction that is large enough to cause ischemic remodeling, but not so large that it causes cardiac arrest. We expect some losses as we optimize the procedure. Historically, myocardial infarction models in canines have been linked to 30-40% mortality; however, these losses can be prevented by treating the animals with amiodarone and lidocaine, in the immediate preoperative, perioperative, and postoperative period, if needed to treat secondary arrhythmias that could lead to death after the MI. This can be done every 30 minutes until normal rhythm returns. Due to this concern for perceived loss we are estimating a need of 20 animals at this time (20 - 40% of 20 = 12). Prior work in our PVC induced cardiomyopathy model has shown that there is an absolute reduction of 20% of LVEF (60%-40%) over the 12 week period. It is expected that this difference will be similar in the ischemic myocardium. We estimate that the LVEF difference of matched animals is normally distributed with standard deviation of 5% points. Assuming that animals have a true difference in the mean LVEF of 20% points between the ischemic hearts with PVC and without PVCs (Power of 0.80, Type I error probability of 0.05). Due to a 10-20% complication rate from PVCs in an inherently arrhythmogenic model, one additional animal will be added in each group (5 + 1; ischemia with PVC, n=6, ischemia without PVC, n=6). In summary, 20 canines - 8 (40% estimated losses from myocardial infarction) - 12 canines / 2 groups = 6 canines - 1 (10-20% for estimated losses due to arrhythmia in this ischemic model) = 5 animals per group.

There will be expected losses due to the initial ischemic injury, which we estimate at 40% given the magnitude of the myocardial injury that will be required to induce significant global cardiac remodeling. The addition of PVCs to this already decompensated heart will produce further destabilization, and likely lead to more morbidity and mortality. It is important that we are able to show the effects of increased PVC burden in hearts that are already remodeled due to ischemic injury because this is a common clinical condition that has not previously been studied. Unfortunately, the combination of these two models will cause cardiomyopathy, and likely result in some heart failure symptoms, such as coughing, abdominal swelling, heavy breathing, exercise intolerance, blue discoloration of mucus membrane and loss of appetite. We still estimate mortality at approximately 50% as noted above, and including losses due to subsequent heart failure.

The numbers used to estimate losses in these studies is based on prior publications from [redacted], and personal discussions with him. He previously used this ligation and latex injection model to study how myocardial infarction alters autonomic activity in the canine**.**

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.)
►

<u>Echocardiogram.</u> A baseline echocardiogram will be performed prior to the first surgery. Follow up echocardiograms will be obtained every 4 weeks until the animal is euthanized. Echocardiogram will take from 10-20 minutes to obtain all required data. We estimate that each animal will undergo from 6 up to 10 echocardiograms depending on the rate of progression and severity of induced heart failure. This non-invasive procedure is not painful and should not cause any distress to the animal. However, it requires that the animal stands still and possibly lays prone or supine for at least 10 minutes in order to obtain accurate cardiac images. For all follow up echocardiograms, we will attempt to acquire images while dogs are trained to stand still on all four legs and lay prone or supine for 10 minutes. However, if

the animal does not cooperate, we will first attempt to mildly sedate the animal with Acepromazine (0.05-0.1mg/kg) given PO approximately 1 hour prior to the procedure. If this is unsuccessful we will have to perform echocardiogram under general anesthesia with endotracheal intubation. We will use Brevital (6-10mg/kg) IV to effect (or Pentobarbital 30mg/kg, if Brevital is not available). Animals will be intubated, mechanically ventilated and anesthetized with isoflurane 1-3%. After the echocardiogram, they will be allowed to recover from anesthesia in a post-operative recovery cage until able to walk to their run. No analgesics will be necessary due to the non-invasive nature of this procedure.
All animals under anesthetic will be continuously monitored by ECG and arterial pressure.

Blood drawn – We will obtain blood sample in all groups at baseline (pre OP), immediately post procedure, the following morning, and on a monthly basis (as described in the exercise test portion) until the end of protocol. We will obtain no more than 10-15 cc, which represents less than 1% of body weight. We will plan to measure changes in atrial and brain natriuretic peptides as marker for heart failure, and troponin as a measure of myocardial injury. Blood will be drawn from the brachial or jugular veins.

First surgery: Myocardial Infarction, Pacemaker Implantation, and Data Sciences International (DSI) nerve recording device implantation.
Prior to first surgery all animals will have two weeks of Amiodarone given orally or by IV (oral administration is preferred but IV may be required in some instances). The first week, they will receive 9mg/kg PO or IV daily and the second week (week immediately before the surgery) we will reduce the dose to 5mg/kg PO or IV daily. The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM, Penicillin (900,000 units) IM and Famotidine 0.5-1 mg/kg PO prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose
Surgery will be performed under full aseptic technique. We will perform a left lateral thoracotomy incision at the T3-4 intercostal space. The heart will be subsequently exposed via transection and retraction of the pericardial sac. While the epicardial surface is exposed we will implant a bipolar epicardial [redacted] lead in the right ventricular (RV) apex. These leads will be connected [redacted] which will be subsequently implanted in a subcutaneous pocket outside of the rib cage. The leads will be tunneled through the tissue to this pocket so that the entire device can be internalized upon closure of the chest, and device pocket. The device will be activated and leads will be tested prior to closure of the chest and pocket. Furthermore, the device will be activated to allow for monitoring of intrinsic arrhythmias in the postoperative period.
Next we will implant a [redacted] device subcutaneously in canines. This device has three bipolar channels with lead that will be tunneled into the thoracic cavity. The first channel will be implanted along the side the caudal end of the stellate ganglion. The overlying fascia will be partially resected to give exposure, and will then be closed over the top of the leads to insulate and secure it in position beside the stellate ganglion. The second channel leads will be positioned alongside the vagus nerve. We will again pull back the overlying fascia to expose the nerve, position our leads along the side of the nerve and reapproximate the fascia to insulate and protect the interaction between the leads and the nerve. The final channel in these devices is a bipole for epicardial electrocardiograms. One lead will be attached to the anterolateral ventricular surface and the

second lead is attached to the left atrial appendage. All leads will be sutured to the surrounding fascia and muscle layers in multiple locations to stabilize the leads in position.

After completion of the intrathoracic lead implantation devices will be tested and set to record. We will then devote our attention towards the visualization and isolation of the proximal segment of the left anterior descending or the left circumflex artery. We ligate the proximal coronary artery for 5 minutes, and release for 5 minutes. We will then permanently ligate the artery and subsequently inject 0.2-1.0 ml of [redacted] into the coronary artery, as needed to visually obstruct the distal arterial branches. The animals will be subsequently monitored for arrhythmias and given additional boluses of amiodarone (7mg/kg IV over 30 60 minutes) and/or lidocaine (2mg/kg bolus) as needed for excessive PVCs, VT or Atrial Fibrillation (AF). In some cases it may be necessary to administer a defibrillator shock to the epicardial surface of the heart. In this case we will have sterile defibrillator paddles available with an attached device that has been programmed to defibrillate at between 30-50 Joules. Once the animal has been deemed stable, after 20-30 minutes of monitoring we will begin closing the surgical wounds.

The pacemaker and DSI leads will be rechecked for stability, and the devices will be implanted in subcutaneous extrathoracic pockets. Once the devices have been implanted in their respective pockets, and the lead positions have been verified along the heart, stellate, ganglion, and vagus nerve we will begin closing all surgical sites. First the thoracotomy site will be closed with surgical steel wires to hold the ribs together. Then the overlying intercostal muscles and deep muscle layers will be closed with interrupted Vicryl sutures. Once this has been closed a previously implanted chest tube will be used to evacuate all air from the pleural cavity and reinflate the left lung. The skin will then be closed in two layers using running Vicryl suture lines. Finally reinforcing nylon vertical mattress sutures will be placed to hold the wound closed during the initial healing phase. All wounds will be covered with a Vetericyn gel spray to promote faster healing. After closure of the thoracotomy, the two subcutaneous device pockets will be closed in the same fashion.

If the animal begins to experience acute cardiogenic shock as a result of the myocardial infarction we may need to administer epinephrine at a low dose (0.01 mg/kg) every 3–5 min early in resuscitation efforts; high dose (0.1 mg/kg) for repeated dosing. For perioperative hypotension we will initiate norepinephrine drip starting at 0.5mcg/kg/min and titrating as needed up to 2mcg/kg/min. Because of the nature of the transmural infarction epinephrine and norepinephrine are likely to cause increased ectopy, including possible ventricular tachycardia or ventricular fibrillation. To mitigate this risk, all epinephrine boluses will be administered with a prophylactic bolus of amiodarone (2.5mg/kg). Norepinephrine drips will be given under closely monitored conditions and amiodarone will be given if required for arrhythmias.
Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive Buprenorphine 0.01-0.02mg/kg IM.
The canine remains in the post-operative recovery cage until the following morning when they are moved to the standard chain link run with padding and blankets. They are given buprenorphine 0.01-0.02 mg/kg IM twice a day for three days for analgesia. Carprofen (2mg/kg PO) will be given for 7 days after the buprenorphine regimen is completed. Famotidine 0.5-1.0 mg/kg PO or IV can be given as needed for nausea or appetite stimulant (metoclopramide 0.2-0.5 mg/kg IV or bismuth subsalicylate 262 mg PO, can be given if needed). Some canines are too active post operatively which prevents incision healing. In this case, they are given Diazepam 0.2-2mg/kg IM or PO twice a day as needed. Cefpodoxime 5mg/kg PO is given once a day for 10 days to prevent wound infection. If there is ongoing concern for wound infection while receiving cefpodoxime (Baytril 5-20mg/kg PO can be given once a day for 10 days). IV Fluids are available with or without 5% dextrose if an animal has not resumed normal eating habits within 2 days. . Canine weights will be observed and recorded daily while on antibiotics and then twice weekly thereafter. Furosemide will be given, if the animal is noted to have

worsening shortness of breath with daily activities, and has visible abdominal wall pitting edema with recorded weight increase greater than 10% of body weight.

They will also receive Amiodarone PO (IV is available if needed) 5mg/kg daily for 7 days post operatively.

Some subjects will be transferred to protocols # 02289 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 protocol #02289. 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. Subjects will be returned to this protocol baring any physical impairments mentioned above, after the completion of procedures on protocol #02289.

Pacemaker interrogation. After pacemaker implantation, device will be interrogated every week to assess for appropriate function, evaluating R wave amplitude (amplitude of ventricular signal), pacing thresholds, histograms and percentage of pacing. This will be performed via [redacted] The programmer is an external device similar to laptop that has a "wand". The wand is positioned close to the device and allows pacemaker evaluation and programming. This process is not painful and will not represent any distress to the animal. We do not expect to require any type of restraint perform pacemaker interrogation. Pacemaker interrogation will last from 5 to 20 minutes depending on the findings.

Holter. Some animals will undergo a single cardiac Holter monitor based on the availability of devices and need for further documentation of cardiac electrical activity. These devices will be paced at least 2 weeks after pacemaker implantation and after baseline echocardiogram. Holter will be worn for 24-48 hours. Several patches will be attached to the skin to obtain lead V1, lead I, III, and aVF. Proper preparation of patch position will be made by shaving the area and cleaning with alcohol pads any residual grease or debris that could interfere with proper attachment of patches. The patches will be secured and covered with Tegaderm. The Holter device will be attached to the animal chest with a canine vest specifically designed for Holter monitors. All wires will be concealed from the dog to avoid any damage to the device. As in humans, this procedure is not painful and will not cause any distress to the animal. We do not expect to require any type of restraint to set up the Holter monitor. After the required time is met, Holter will be removed and sent for analysis of PVC burden or percentage, and autonomic activity. We plan to repeat these Holter monitors up to twice weekly throughout the testing period in order to correlate this data with the implanted DSI device.

In our experience there have been no issues with skin irritation from the use of our Holter monitors. However, in the unlikely event that the skin becomes irritated after the Holter monitor is removed, we will postpone any subsequent monitoring phase until after the skin has healed, thus negating the twice weekly monitoring.

Autonomic Nerve recording. We will turn on the DSI to record data on the evening after surgery for the first 24-72 hours. The process of activating the DSI recording device is not painful to the animal and should require no additional medication. The device will record for 24-72 hours, and this process will be repeated weekly during the first month recovery period. We will then turn on pacemaker, as described below in the section titled Chronic Pacing Protocol. DSI devices will be activated for 24-72 hours weekly until the terminal surgery. Diazepam (0.2-2.0 mg/kg PO or IM) can be given for any distress noted.

Training. Most non-surgical procedures (echocardiograms, electrocardiography, pacemaker interrogation and blood drawn) will be performed in a conscious state with minimal or no sedation. In

order to achieve this, all animals will undergo training in order to lay or sit down still from 20-30 minutes at a time. This training will be performed by technicians. We estimate that this training will take from 2-4 weeks. Methods used for training will consist mostly on repetition with reward after completing different tasks, which will be gradually introduced and increased the duration of time until animals can lay or sit down for at least 30 minutes.

Cardiomyopathy model. Chronic Pacing protocol. To prevent symptomatic congestive heart failure but still allow for development of cardiomyopathy, the ventricular pacing protocol will be performed over 12 weeks of Bigeminy 200 PVCs. This typically allows for the gradual development of cardiomyopathy without symptomatic congestive heart failure. Acepromazine (0.05-0.1mg/kg) PO and Buprenorphine 0.01-0.02mg/kg IM will be administered as needed for distress.

Treadmill. Canines will be exercised on a DogPACER (canine specific treadmill) to observe how PVCs are affecting their exercise capacity, autonomic nervous recordings and possible arrhythmias. This procedure will be performed at the same time as the follow up echocardiograms: one month post operation, and each subsequent month during the Chronic Pacing Model until the terminal surgery. To acclimate the animals to the treadmill, they will initially be introduced by letting them explore the exercise room and equipment until they have become comfortable with those surroundings. Presence of normal, relaxed behavior will signal that the dogs are ready for the next step, which is putting them on the treadmill while it's off. This will occur in small steps, putting them on for seconds and then extending the time. Each positive reaction will be rewarded with treats to encourage the dogs' learning process. When the dogs have become relaxed with the task of being on the still treadmill, they will next be put on the treadmill at its slowest speed, 0.5 mph. Two people will assist in this process; one person will hold the leash of the dog and stand in front of the treadmill offering rewards for positive behavior while the other will stand behind the animal making sure that she does not slide off of the machine, jump off of the sides and also to help the dog move their feet until she begins to understand and be comfortable with the movement herself. The process will take as long as needed to have the dogs become comfortable with the treadmill**.**

Each workout lasts 9 minutes, in which the dogs will complete 3 stages, each lasting 3 minutes. The first setting will be 1.1 mph followed by 3 minutes at 2.2mph and 3 more minutes at 3.3 mph An average dog can run 20-30mph therefore the speeds in this procedure result in a slow jog/fast walk. We will determine fitness based on heart rate and serum lactate acid. Heart rate will be recorded before, at the peak of workout and at the finish. At that point, heart rate will be recorded every minute until it returns to baseline. The amount of time it takes for heart rate to return to baseline post-workout is the true measure of fitness. Heart rate can be displayed and monitored during the workout by a pacing analyzer that connects wirelessly to pacemaker implanted. In this way, we can also assess arrhythmias as the mild cardiomyopathy develops.

Blood samples will be obtained through an IV catheter placed in the brachial or jugular veins. Blood will be drawn 3 times: once before the study and recovery phase without and with PVCs, without exceeding 15mL (less than 1% of animal's body weigh). The blood will be drawn up through a syringe connected to a sterile intravenous catheter put in the brachial or jugular veins. Each subsequent blood draw will come from a new clean IV, as the animals seem to tolerate this better than a line that is left in place. If necessary because of a dog's personality, Acepromazine (0.05-1.0 mg/kg PO) will be used to put the IV catheter in place effectively. After monitoring is done, the blood will be spun in a centrifuge and samples stored in -80 degrees Celsius until study at a later date.

We have found that PVCs induced a mild cardiomyopathy, and yet, we cannot see physical findings of heart failure. For that reason, we need to be able to assess heart failure or decrease in exercise capacity in the canines. The true definition of heart failure states that symptoms are present at extreme or high levels of exertion. All animals thus far appear to be class I HF, but technically some of them may start at class I and later transition into class II HF.

Intravenous pharmacological challenge. In order to understand and validate recordings in the stellate and vagal nerve, we plan to pharmacologically stimulate autonomic nerves by administration of short-acting intravenous vasoactive drugs (clonidine or phenylephrine) one month post operation, and each subsequent month during the chronic pacing model until the terminal surgery.

The drugs used are ones commonly used in clinical practice in humans but have been also used in canines. They are all short-acting drugs whose half-lives do not exceed 12 hours when given orally. Therefore, when administered IV, their effects peak within minutes and half-lives usually less than 4 hours as described below:

1. IV clonidine (10 µg/kg) is an alfa-2 agonist which will suppress central sympathetic nerve discharge by acting on pre-synaptic alfa-2 receptors in sympathetic nerve terminals. IV Clonidine peaks within an hour and has a plasma half-life of 2-3 hours. Blood pressure and heart rate are expected to drop but the doses used have been reported in the literature [Cavero, Br. J Pharmacol 1980; 70:269]. We expect that the effects are transient and will not have long term sequelae.

2. IV phenylephrine 0.01mg/kg. Phenylephrine is a vasopressor used to increase BP. This will be given as an IV bolus. The increase in BP will suppress sympathetic nerve activity and potentiate vagal nerve activity. We expect that the effects are transient and will not have long term sequelae. [Varma S, Circulation Research 1960;8:1182].

The animals will be challenged twice with each of the above drugs. The drugs will be challenged one at a time on a separate day each.

We will maintain a log of blood pressure readings, heart rate, time of administration and physical characteristics during the monitoring phase and keep this information in the animals folder for review.

PVC challenge and Ventricular Programmed Stimulation.

PVC challenge. PVCs are administered through the implanted cardiac device. The purpose is to determine the response of autonomic nerve activity to isolated PVCs administered for 2 minutes at different frequencies (at 200ms coupling interval at bigeminy, trigeminy, quadrigeminy) and then in bigeminy at different coupling intervals (200-350ms). The test is helpful to determine the mechanism by which autonomic nerve activity is triggered by PVCs. Understanding this mechanism is important in devising future therapeutic strategies aimed at preventing dysautonomias caused by PVCs. The PVCs are administered at twice the diastolic threshold for 2 minutes at a time, and then disabled for 2 minutes, and then reapplied again until the test is completed.

Ventricular programmed stimulation (VPS) will also be performed through the implanted cardiac device The purpose is to determine ventricular effective refractory period (VERP) and test susceptibility of ventricular arrhythmias. For VERP, a train of S1 at 400 and 300 ms cycle length is followed by S2 with 10 ms-decrement until S2 is unable to capture. Stimulus strength is twice the diastolic threshold. VERP is defined as the longest S1-S2 interval that does not elicit ventricular capture. For the evaluation of susceptibility to ventricular arrhythmias and drug effects, two 10-beat S1 trains at 400 and 300 ms cycle length with consecutive extra stimuli (S2 and S3) each with a gradual 10 ms-decrement until loss of capture is noted. VPES will be performed in a non-sedated state at baseline, after completion of PVC protocol (PVC group).

All animals will undergo VPS at baseline (2 weeks post-thoracotomy), and 1-7 days prior to final surgery. All animals will undergo PVC challenges 4 weeks post-surgery before PVCs are started, monthly post PVC's on, at the end of the 12 week PVC period, and after 4 weeks of PVC recovery.

If sustained ventricular arrhythmias are induced, an external defibrillator and epinephrine will be in the prep room in order to resuscitate animal and restore normal rhythm. If defibrillation is indeed needed, animal will receive Carprofen 2mg/kg daily for 1-3 days. If epinephrine is administrated it will be

administrated via IV at a low dose (0.01 mg/kg) every 3–5 min early in resuscitation efforts; high dose (0.1 mg/kg) for repeat dosing. Amiodarone (7mg/kg) will be used as an alternative defibrillation. This is administrated in a single dose and repeated as needed every 30 minutes.

Terminal surgery The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose

A 4th or 5th intercostal thoracotomy will be performed. Basic electrophysiologic studies will be performed in vivo, including measurement of effective refractory period, monophasic action potential and standard programmed stimulation protocols, including measurement of atrial effective refractory period.

In order to most accurately view autonomic nerve activity during an EP study, isoflurane will be turned off for a period of about 30-45 minutes. During this time, pentobarbital will be administered as follows to keep the animal in a surgical plane of anesthesia. We will give 4-5mg/kg of pentobarbital initially and then turn off the isoflurane. The heart rate will be monitored closely. Average heart rate for the dogs under anesthesia is 85-100bpm. If heart rate increases by more than 5bpm from the average to this point, small doses of pentobarbital (2-3 mg/kg not to exceed a total 30 mg/kg including the loading dose) will be administered to effect. Once the nerve recording is finished, isoflurane will be resumed until end of surgery. Isoflurane has a nature of suppressing nerve activity and does so more than pentobarbital. For this reason, isoflurane is removed. If the maximum dose of pentobarbital is reached during surgery, isoflurane must be resumed and the EP study completed under the effects of pentobarbital.

Blood will be collected from coronary sinus as well as aorta by direct sampling. The dog will be exsanguinated by removal of the heart under anesthesia. Tissues will be collected and preserved in 4% formaldehyde for 1 hour, before being stored in 70% alcohol. Additional tissue will be snap frozen in liquid nitrogen and stored for histopathology and molecular biology.

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

►► Canines have very similar physiology to humans. In addition there are significant differences in cardiac physiology between small animal species and humans. The experimental techniques, electronic pacemakers and leads available are large and require a larger species. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated large [redacted] **electronic defibrillator / pacemaker, which has been specifically developed for our study. The radiotelemtry device is also large and will require internal implantation and observation for several months. Mostly biological pacemakers have been developed in smaller, less sentient species. In contrast to the electronic defibrillator / pacemaker, the biological pacemaker cannot modified its behavior easily, store and analyzed data. Moreover, an animal model with dogs has also been extensively studied in tachycardia-induced cardiomyopathy using an electronic pacemaker. Additionally, dogs have a His-Purkinje system located in endocardium, very similar to the human's heart, which pigs and other larger animals do not have.**

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
First Surgery	[redacted]
Pacemaker interrogation	[redacted]
Autonomic Nerve recording.	[redacted]
Echocardiogram	[redacted].
Blood drawn	[redacted]
Electrocardiogram	[redacted]
Intravenous pharmacological challenge	[redacted].
Ventricular programmed stimulation (VPS)	[redacted].

Treadmill Exercise Challenge	[redacted]
Cardiomyopathy model. Chronic Pacing protocol	[redacted]
Terminal/Final Surgery	[redacted]

Co-PI:
Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
First Surgery	[redacted]
Pacemaker interrogation	[redacted]
Echocardiogram	[redacted]
Blood drawn	[redacted]
Electrocardiogram	[redacted]
Final/ Terminal Surgery	[redacted]

► [redacted]
► [redacted]

[redacted]

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
First Surgery	[redacted]
Pacemaker interrogation	[redacted]
Autonomic Nerve recording.	[redacted]
Echocardiogram	[redacted]
Blood drawn	[redacted]
Electrocardiogram	[redacted]
Intravenous pharmacological challenge	[redacted]
Ventricular programmed stimulation (VPS)	[redacted]
Treadmill Exercise Challenge	[redacted]
Cardiomyopathy model. Chronic Pacing protocol	[redacted]
Terminal/Final Surgery	[redacted]

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 this individual will perform	Experience with each procedure in the species described in this ACORP
First Surgery	[redacted]
Final/ Terminal Surgery	[redacted]

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

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP

First Surgery	[redacted]
Terminal/Final Surgery	[redacted]

Name▶ [redacted]
Animal research experience ▶ [redacted]
Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
First Surgery	[redacted]
Pacemaker interrogation	[redacted]
Autonomic Nerve recording.	[redacted]
Echocardiogram	[redacted]
Blood drawn	[redacted]
Electrocardiogram	[redacted]

Intravenous pharmacological challenge	[redacted]
Ventricular programmed stimulation (VPS)	[redacted]
Treadmill Exercise Challenge	[redacted]
Cardiomyopathy model. Chronic Pacing protocol	[redacted]
Terminal/Final Surgery	[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]	
[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	

[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	

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"
►

[redacted]

No other investigators will require training for these procedures.

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]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]			[redacted]
[redacted]	[redacted]			[redacted]

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	female	20-30kg	[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►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**

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►VPS, Myocardial Infraction						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**
Canine infarct with PVC	6	4				10
Canine infarct without PVC	6	4				10

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
Canine infarct with PVC	6	4				10
Canine infarct without PVC	6	4				10

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

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

► (X) 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)
First Surgery	See App 5	[redacted]	See app 5

Final/Terminal Surgery	See App 5	[redacted]	See app 5

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

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

► (X) 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.

►

Myocardial Infarction

The side effects of having a large myocardial infarction are chest pain, shortness of breath, and occasionally nausea. We are able to ameliorate the symptoms of the myocardial infarction with analgesics but full alleviation is not possible. These symptoms typically resolve within 3-5 days. Opiods and NSAIDs are given throughout this timeframe. Additionally, the size and full muscle thickness of the expected myocardial injury will make the hearts electrically unstable which may lead to ventricular tachycardia (VT), ventricular fibrillation (VF) and even death in up to 40% of animals. We will minimize the chance of these occurring using several agents (i.e. preoperative and postoperative prophylactic amiodarone) and analgesics and monitor for arrhythmias using the implanted devices.

There is a chance that animals will develop an arrhythmia during the postoperative period that will require treatment with defibrillation by internal or external device. We will provide analgesics to reduce the pain and distress from this process but it cannot be fully alleviated.

Ventricular Programmed Stimulation. Ventricular programmed stimulation (VPS) will be performed through the implanted cardiac device to determine ventricular effective refractory period (VERP) and test susceptibility of ventricular arrhythmias. The arrhythmias will lead to mild discomfort in the form of 'heart fluttering or racing' that may be distressful to the animal. There is no analgesic or anesthetic to alleviate this feeling and sedatives can interfere with the arrhythmias. This feeling will last approximately 3 seconds. If sustained ventricular arrhythmias are induced an external defibrillator will need to be used to restore normal rhythm. If this is used, carprofen will be administered 2mg/kg for 1-3 days afterward however this will not completely alleviate the pain from the defibrillation.

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]
   Institutional affiliation►[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 for the animal husbandry staff, 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:

[redacted]	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	Chain link run, 3x6 and 4x10 feet cage	1	no	3

*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:
► **Chain link run, 3x6 feet cages**

** 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 with one another since each room has two to five dog runs. In addition, while their runs are being cleaned on a daily basis, pairs of dogs are allowed to exercise and play together in a designated "romper room". Animals are fitted with DSI transmitters and need to be housed singly in a cage for which DSI receivers are installed to receive signals from the transmitters. Mixing dogs will result in data cross talk.**

***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**	**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.
►**Per SOP, dogs can see, smell and interact with each other through the chain link. They are provided 10-20 minutes of interaction with the animal caretaker daily and toys/treats are provided and 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.
►

► (X) 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.
►**This pertains to cage cleaning and letting the animal out of the cage. To avoid cross talk between different transmitters being picked up by a receiver, the dogs should only be let out one at a time, and at a pre-specified time (so it is clear what period of time data will be lost or there is potential cross talk).**

After the initial surgery, which includes the myocardial infarction procedure, animals will be monitored for early symptoms of heart failure. They will continue to be monitored with daily to twice daily checks for wellbeing for the remainder of the study. If the animals are noted to have convincing signs of heart failure, such as coughing, abdominal swelling, heavy breathing, exercise intolerance, blue discoloration of mucus membrane and loss of appetite ,animals will be transitioned to a low sodium diet. We do not expect to see these symptoms in the first 30 days post surgery. Initial signs would most likely be noted during the first exercise challenge.

► ( ) 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.

► ( ) 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
First Surgery	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Terminal surgery	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Blood Draw	**( )**	**(X)**	[redacted]	**( )**	**(X)**
Echocardiogram	**( )**	**(X)**	[redacted]	**( )**	**(X)**
Drug Challenge		**(X)**	[redacted]	**( )**	**(X)**
Treadmill		**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	Collected AFTER Euthanasia	Collected BEFORE 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")
Blood from coronary sinus and aorta	**( )**	**( )**	**(x)**	**( )**
Heart	**(x)**	**( )**	**( )**	**( )**
Pacemaker and Data Sciences International (DSI) device.	**(x)**	**( )**	**( )**	**( )**

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

► (x) 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".

► ( ) 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.)

►Intraoperative complications that could result in euthanasia are vessel puncture that results in more than a 20% blood loss, or an extreme myocardial infarction as a result from injecting the latex dye that causes the animal to have complete heart failure.

When the animal begins to show initial signs of heart failure, as defined above, the veterinarian will be immediately notified for increased monitoring. Animal charts will be updated to reflect this notification. If animals suffer from severe heart failure symptoms that are not controlled with diuretics and reduced sodium diet after 2 weeks, they will satisfy end point and euthanasia will be performed. Symptoms will include: increased heart rate/anxiety, weight loss of more than 12%, not eating, lethargy, weight gain due to fluid retention, signs of fluid accumulation in prone locations (AKA pitting edema, and in the case of canines abdominal ascites), coughing, exercise intolerance, blue discoloration of tongue and mouth and increased general fatigue. An attempt will be made to treat these animals with furosemide 0.2-0.5mg/kg (IV or PO, but if the animals do not recover to the point of normal appetite within one week they will satisfy end point and euthanasia will be performed. Weights will be monitored twice a week starting after the first surgical procedure and continued until the animal completes the protocol. If weights drop more than 5%, the veterinarian will be notified and weights will be measured daily until completion of protocol or until weight returns to normal. This weight will be logged in the animals file

Furthermore, if animals have abdominal distention from ascites that does not resolve with 2 weeks of diuretic treatment they will satisfy end point and euthanasia will be performed.

As a part of the study, all animals will undergo exercise treadmill testing. In the event that the animals cannot tolerate a modified stress test, consistent with class 2 heart failure (i.e. exercise intolerance), we will continue to monitor the animals, and treat with diuretics, beta blockers, and nitrates as above. For animals noted to have this symptom, treadmill test will be discontinued until the symptom has resolved via medical therapy and reduced sodium diet as noted above. However, euthanasia will be considered if they develop class 4 heart failure (persistent inability to eat or shortness of breath at rest or with daily activities) that does not improve with the aforementioned medical therapy.

Blood pressure will also be monitored weekly and recorded in the chart to be sure animals do not become hypotensive. However, in our experience, this has not occurred. If animals have symptomatic hypotension causing loss of consciousness, or dizziness that makes them unable to remain steady on their feet long enough to walk down the hallway they will satisfy end point and euthanasia will be performed.

If the canine was to suffer a serious postoperative complication such as uncontrollable bleeding, pneumothorax, renal infarction (presenting as hematuria, pain, renal failure manifested by oliguria, anuria, fatigue, listlessness) as a result of occlusion of renal artery they will satisfy end point and euthanasia will be performed.

If there is uncontrollable infection of the pocket in which pacemaker and DSI are placed, and these cannot be controlled by antibiotics or washout they will satisfy end point and euthanasia will be performed. Pocket infection presents as pocket swelling with purulence, erythema, pain, fever, anorexia, which does not resolve with drainage and/or antibiotics. Note that pocket seroma (a pooling of non-infectious fluid in the device pocket) is common after surgery but this presents merely as pocket swelling without inflammatory features as above.

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

► ( ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by

these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

►

► (X) 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►		( )	( )	( )
(X)	Anesthetic overdose Agent► Pentobarbital Dose►100 mg/kg Route of administration►IV		(X)	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )

(X)	Exsanguination under anesthesia Agent► Brevital and Isoflurane (maintenance with intubation) Dose►6-10 mg/kg (brevital) and 1-3% (isoflurane) Route of administration►IV		(x)	( )	( )
( )	Other (Describe) ►		( )	( )	( )
( )	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.
   ► **Drs** [redacted] **and** [redacted] **will perform euthanasia. All have extensive experience in canine surgery of the nature described for second surgery.** [redacted]

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.
   ► [redacted] **The carcass should be kept in a refrigerator. A postmortem examination will be performed and the hearts harvested for histopathology and storage.**

   b. Describe how the PI's staff should be contacted.
   ► ( ) 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
Drug Challenge		Items:C.2.c	(x)**
Radio Telemetry		Items: C.2.c	(x)**
Treadmill Exercise		Items: C.2.c	(x)**
Echocardiogram		Items: C.2.c	(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.
   ► Myocardial infarction, thoracotomy, heart failure, and chronic PVC induced left ventricular dysfunction

   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.

					Indicate which mandate each search addressed			
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	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)
Medline	5/15/2017	2006-2017	myocardial infarction Left ventricular dysfunction and PVCs. Autonomous nerve activity in PVCs	transmural myocardial infarction, ischemic cardiomyopathy, thoracotomy, heart attack, remodeling Premature ventricular contractions, cardiomyopathy, LV dysfunction, thoracotomy, heart attack, remodeling	(X)	(X)	(X)	(X)
ALTWEB	5/15/2017	2006-2017	myocardial infarction Left ventricular dysfunction and PVCs, Autonomous nerve activity in PVCs.	transmural myocardial infarction, ischemic cardiomyopathy, thoracotomy, heart attack, remodeling Premature ventricular contractions, cardiomyopathy, LV dysfunction,	(X)	(X)	(X)	(X)

				thoracotomy, heart attack, remodeling				

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 study of changes in LV function and cardiac contractility due to frequent PVCs in the ischemic heart is unknown and therefore, there are no computer models to answer the unknown questions. Our lab has recently described the first animal model of PVC-induced cardiomyopathy in canines. Otherwise, there are only studies in humans in whom frequent PVCs appear to affect cardiac contractility, however, there are multiple uncontrolled variables and thus clinical studies have multiple limitations.

The experimental techniques, electronic pacemakers and leads available are large and require a larger species, therefore smaller animals such as mice and rabbits are not appropriate. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated large [redacted] **defibrillator / pacemaker, which has been specifically developed for our study. This device will require internal implantation and observation for several months. Mostly biological pacemakers have been developed in smaller, less sentient species. In contrast to the electronic defibrillator / pacemaker, the biological pacemaker cannot modified its behavior easily, store and analyzed data. Moreover, an animal model with dogs has also been extensively studied in tachycardia-induced cardiomyopathy using an electronic pacemaker, since dogs have a His-Purkinje system located in endocardium, very similar to the human's heart.**

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.
►
The number of animals has been reduced to the minimum without compromising results as outlined in the power size estimation. In addition all data for subsequent Aims (2 and 3) will be obtained in parallel to reduce the number of animals required to complete this study.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
►
Our project is design to minimize pain and distress, as well as use as few animals as possible. We will follow AWA recommendations to minimize distress and pain. Animals will have at least a week to acclimate to the new environment. Postoperatively, animals will receive analgesics and close monitoring to assess for any signs of pain or distress, such as weight loss, lethargy, limping, vocalizing, excessive licking and even aggression. In addition, animals will be trained by technicians to undergo pacemaker interrogation and echocardiogram with minimal distress. The dogs will be handled daily to allow for maximum comfort and enjoyment of their environment.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
►
Review of literature shows that this proposed study is novel and has never been performed previously.

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
buprenorphine	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Pentobarbital	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Brevital	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Diazepam	X	X	[redacted]	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"
► (x) Appendix 5, "Surgery"
► (x) 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
[redacted]	[redacted]	[redacted]

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

1. **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
Pentobarbital	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
[redacted]	[redacted]	( )	( )BSL_	( )	( )	( )	( )	( )
Pacemaker/ICD	[redacted]	( )	( )BSL_	( )	( )	( )	( )	( )
Clonidine	Richmond VA pharmacy	(X)	( )BSL_	( )	( )	( )	(X)	( )
Metoclopramide	Richmond VA Pharmacy							
Phenylephrine	Richmond VA pharmacy	(X)	( )BSL_	( )	( )	( )	(X)	( )
Isoflurane	Richmond VA pharmacy	( )					(X)	
Brevital	Richmond VA pharmacy	( )					(X)	
Buprenorphine	Richmond VA pharmacy	( )					(X)	

Diazepam	Richmond VA pharmacy	( )					(X)	
Acepromazine	Butler Schein	( )					(X)	
Carprofen	Butler Schein	( )					(X)	
Famotidine	Local pharmacy						(X)	
Meloxicam	Butler Schein						(X)	
Epinephrine	Richmond VA pharmacy						(X)	
Amiodarone	Richmond VA pharmacy						(X)	
Lidocaine	Richmond VA pharmacy						(X)	
Blue latex dye	Carolina						(X)	
Levophed (norepinephrine)	Richmond VA pharmacy						(X)	
furosemide	Richmond VA pharmacy						(X)	
Vetericyn Gel Spray	Butler Schein						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)
Acepromazine	0.05-0.1mg/kg		IV or PO	Once for each procedure	sedative	App5	Y
Pentobarbital–survival surgery or euthanasia	30mg/kg total (to effect) or 100mg/kg for euthanasia		IV	twice per surgery or once for euthanasia	Anesthetic	App5	Y
Baytril	5-20 mg/kg		PO	10 days	Antibiotics	App5	N
Buprenorphine	0.01-0.02 mg/kg		IM	Q8-12hr	Analgesics	App5	N
Data Sciences international device			SC implantation during first surgery	Once	study device	App5	Y

Pacemaker device			SC implantation during first surgery	once	study device	App5	Y
Clonidine	10 µg/kg	Dextrose 5%	IV	Twice in 3 months (1st at week 2 post-op & 2nd after week 8)	Assess correlation between renal and cardiac sympathetic nerves. Transient (minutes) drop in BP, HR	Pg 23	Y
Metoclopromide	0.2-0.5 mg/kg		IV or PO	As needed	Antiemetic	App5	N
Phenylephrine	0.1mg	Dextrose 5%	IV	Twice in 3 months (1st at week 2 post-op & 2nd after week 8)	Assess correlation between renal and cardiac sympathetic nerves. Transient (minutes) drop in BP, HR	Pg 23	Y
Brevital	6-10 mg/kg to effect	Normal saline	IV	Once for 1 day	Sedation during surgery	App5	Y
Diazepam	0.2-0.4 mg/kg		PO or IM	Once a day as needed	Calm during post-op to keep sutures intact	App5	N

Carprofen	2 mg/kg		Oral	SID for 7 days	Anti-inflammatory and pain relief during post-op	App5	N
Isoflurane	1-4%		Inhalation	Continuous during surgery	Sedation during surgery	App5	Y
Penicillin	3 ml (300,000 units/ml)	None	IM	Once for 1 day	Antibiotic, prevent infection	App5	N
Bismuth Subsalicylate	262 mg	None	Oral	Once a day as needed	Appetite recovery	App5	N
Famotidine	0.5-1.0mg/kg	none	Oral	Once a day as needed	Appetite recovery	App5	N
Meloxicam	0.2 mg/kg	none	IM or SQ	Once or as an alternative for Carprofen	Pain Relief	App5	N
Epinephrine	Low dose (0.01 mg/kg) high dose (0.1 mg/kg)	Normal Saline	IV	every 3–5 min early in resuscitation efforts; after prolonged resuscitation efforts (15 mins)	Resuscitation efforts	App5	Y
Amiodarone	Oral: 9mg/kg loading week 1 and 5mg/kg wee before surgery and week after surgery	5% dextrose in water for IV form	Oral tablet for prophylaxis and IV bolus for arrhythmia or prophylaxis during	Oral dose daily, and IV dose during surgery repeated every 30 minutes if necessary	Oral: prophylactic prevention of arrhythmia, IV: Resuscitation	App5	Y

	maintenance, IV 2.5-5mg/kg for arrhythmias		shock					
Levophed (norepinephrine)	IV drip initiated at 0.5mcg/kg/min with titration up to 2mcg/kg/min or higher	Normal saline	IV	Given as a drip perioperatively	IV	App5	Y	
Lidocaine	1-2mg/kg	None	Bolus	1-2mg/kg/hr infusion	treat arrythmias	App5	Y	
Blue latex dye (Carolina biological supply)	0.2-2.0 ml	None	Inter arterial Bolus, directly to coronary artery	once during initial surgery	induce myocardial infarction	App5	Y	
Furosemide	0.2-0.5mg/kg	None	IV or PO	Given daily or twice daily	To alleviate congestive heart failure symptoms	App 6	N	
Vetericyn Gel Spray	2-3 sprays	None	Topical	Once after surgery	Promote wound healing	Main body	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 agents used will be pharmaceutical grade except for blue latex which is not commonly used in medical practice. We use [redacted] **these experiments because it is designed for experimental injections. It is necessary to create a complete occlusion of the coronary artery bed, and prevent back flow for the extensive collateral network in the canine heart. The dye is poured into a sterile conical tube prior to the surgery. Upon opening the container initially prior to surgery, a drop will be put into sterile media and incubated to ensure sterility of the product.**

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

a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):

► Acepromazine (0.5-2.0 mg/kg PO) is given prior to the Brevital (or Pentobarbital) administration via IV catheter. The animal is sedated with Brevital or Pentobarbital prior to isoflurane administration. The DSI device and Pacemaker are implanted under isoflurane anesthesia and the latex dye is injected under isoflurane anesthesia.

b. 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.

► All agents given without anesthesia are administered via IM, IV or oral routes. Injections require no anesthesia as only momentary pain is experienced and none of the agents are irritating to the tissues. Agents given orally can be hidden in treats provided by the VMU or provided in a flavor tab that is eaten voluntarily by the canine.

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	a. Mutagen	b. Carcinogen	c. Teratogen	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	
Clonidine	( )	( )	( )	(X)	( )	( )*	(X)►anti-hypertensive
Phenylephrine	( )	( )	( )	(X)	( )	( )*	(X)►hypertensive
Blue latex dye	( )	( )	( )	(X)	( )	( )*	(X)►intravascular occlusion

*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
		(Yes/No)	( )	( )	( )**
		(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

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

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
	( )	( )
	( )	( )

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
Clonidine	Transient (minutes) drop in BP, HR	Acepromazine is given and the drugs are very short lived
Phenylephrine	Transient (minutes) increase in BP	Acepromazine is given and the drugs are very short lived
Blue latex dye	Will occlude coronary artery causing acute chest pain with some lasting distress	Drug will be administered while dog is in anesthetic plane, and analgesia will be maintained postoperatively with buprenorphine, and later in the recovery period with Carprofen and meloxicam

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
Clonidine	SRS	VA	No staff members at risk
Phenylephrine	SRS	VA	No staff members at risk
Blue latex dye	SRS	VA	No staff members at risk

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.
►No staff members will be at risk.

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

ACORP Appendix 4
ANTEMORTEM SPECIMEN COLLECTION
VERSION 4

See ACORP App. 4 Instructions, for more detailed explanations of the information requested.

1. **Summary.** Complete the table below for each specimen to be collected from a live animal on this protocol (see ACORP App. 4 Instructions, for details).

Specimen Collected	Site and Method of Collection	Anesthesia (Yes/No)	Amount Collected Each Time	Volume Replacement (Yes/No/NA)	Total Number of Collections per Animal	Time Intervals Between Successive Collections
Blood	**Brachial or Jugular vein/ Phlebotomy**	**No**	**5-15 ml (< 1%)**	**No**	**5**	**Monthly**
Blood	Coronary Sinus/Aorta	Yes	<10mls	Yes	2	First surgery to final surgery

2. **Use of Anesthetics, Tranquilizers, or Analgesics**.

a. For each specimen described in Item 1, above, as being collected WITHOUT anesthesia, complete Items 2.a(1) and 2.a(2), below:

(1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain).
►The collection method for the brachial involves no more than minor or momentary pain.

(2) Completely describe any method of physical restraint that may be used.
►

b. For each specimen described in Item 1, above, as being collected WITH anesthesia, complete the following table:

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) and volume (ml)	Route of administration	Frequency of administration
Acepromazine	**0.05-1.0 mg/kg**	**Oral**	**Once**
Brevital	6-10 mg/kg	IV	Once
Isoflurane	1-3%	Inhalation	continuous

3. **Volume Replacement for Fluid Collections.**

a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.

►The volume is small and replacement is not required.

b. For each fluid specimen described in Item 1, above, for which volume replacement WILL be provided, describe the replacement fluids that will be administered (including their composition, volume, and route of administration).

► During surgery, they are on a constant IV fluid drip.

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).

► [redacted] monitor the dogs 5-10 minutes after blood draw to ensure they have recovered. The blood draws during surgery will follow the post operative monitoring regimen described in Appendix 5.

ACORP Appendix 5
SURGERY
VERSION 4

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	Initial/First Surgery	( )	( )	(X)	(X)*
2	Terminal/ Final Surgery	(X)	( )	( )	(X)*
3	Lead Revision			(X)	(X)
4	Wound Repair		(X)		(X)

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:

►
Lead Revision.This would only occur in the unique circumstance in which the pacemaker lead has unintentionally moved/dislodged. If this were to occur, animal will not provide scientific data for research, thus we believe that in some circumstances it would justify a second survival surgery to reposition the lead and have appropriate pacemaker function that would provide proper data for analysis.
Wound revision- it is not uncommon for surgical wound dehiscence to occur immediately after surgery. A minor and quick procedure would be needed to correct any defect.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
► Lead Revision- A minimum of 14 days to 8 weeks between the initial surgery and lead revision. This surgery will not be performed before the animal's incisions have fully healed.

Wound revision- This would occur 2-7 days after the initial surgery if needed.

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

1. First surgery: Myocardial Infarction, Pacemaker Implantation, and Data Sciences International (DSI)

nerve recording device implantation.

The canine will receive Amiodarone for two weeks prior to surgery. For the first week, they will receive 9mg/kg PO daily(IV is available if needed) and the second week (the week immediately prior to surgery) the dose will be reduced to 5mg/kg PO daily.
The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM, Penicillin (900,000 units) IM and Famotidine 0.5-1 mg/kg PO prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose

Surgery will be performed under full aseptic technique. We will perform a left lateral thoracotomy incision at the T3-4 intercostal space. The heart will be subsequently exposed via transection and retraction of the pericardial sac. While the epicardial surface is exposed we will implant a bipolar epicardial [redacted] (RV) apex. These leads will be connected [redacted] which will be subsequently implanted in a subcutaneous pocket outside of the rib cage. The leads will be tunneled through the tissue to this pocket so that the entire device can be internalized upon closure of the chest, and device pocket. The device will be activated and leads will be tested prior to closure of the chest and pocket. Furthermore, the device will be activated to allow for monitoring of intrinsic arrhythmias in the postoperative period.
Next we will implant a [redacted] device subcutaneously in canines. This device has three bipolar channels with lead that will be tunneled into the thoracic cavity. The first channel will be implanted along the side the caudal end of the stellate ganglion. The overlying fascia will be partially resected to give exposure, and will then be closed over the top of the leads to insulate and secure it in position beside the stellate ganglion. The second channel leads will be positioned alongside the vagus nerve. We will again pull back the overlying fascia to expose the nerve, position our leads along the side of the nerve and reapproximate the fascia to insulate and protect the interaction between the leads and the nerve. The final channel in these devices is a bipole for epicardial electrocardiograms. One lead will be attached to the anterolateral ventricular surface and the second lead is attached to the left atrial appendage. All leads will be sutured to the surrounding fascia and muscle layers in multiple locations to stabilize the leads in position.

After completion of the intrathoracic lead implantation devices will be tested and set to record. We will then devote our attention towards the visualization and isolation of the proximal segment of the left anterior descending or the left circumflex artery. We ligate the proximal coronary artery for 5 minutes, and release for 5 minutes. We will then permanently ligate the artery and subsequently inject 2-3ml of Blue latex (Carolina Biological Supplies) into the coronary artery, as needed to visually obstruct the distal arterial branches. The animals will be subsequently monitored for arrhythmias and given additional boluses of amiodarone (2.5-5mg/kg IV over 30 60 minutes) and/or lidocaine (2mg/kg bolus) as needed for excessive PVCs, VT or AF. In some cases it may be necessary to administer a defibrillator shock to the epicardial surface of the heart. In this case we will have sterile defibrillator paddles available with an attached device that has been programmed to defibrillate at between 30-50 Joules. Once the animal has been deemed stable, after 20-30 minutes of monitoring we will begin closing the surgical wounds.

The pacemaker and DSI leads will be rechecked for stability, and the devices will be implanted in

subcutaneous extrathoracic pockets. Once the devices have been implanted in their respective pockets, and the lead positions have been verified along the heart, stellate, ganglion, and vagus nerve we will begin closing all surgical sites. First the thoracotomy site will be closed with surgical steel wires to hold the ribs together. Then the overlying intercostal muscles and deep muscle layers will be closed with interrupted Vicryl sutures. Once this has been closed a previously implanted chest tube will be used to evacuate all air from the pleural cavity and reinflate the left lung. The skin will then be closed in two layers using running Vicryl suture lines. Finally reinforcing nylon vertical mattress sutures will be placed to hold the wound closed during the initial healing phase. All wounds will be covered with Vetericyn gel to promote faster healing. After closure of the thoracotomy, the two subcutaneous device pockets will be closed in the same fashion.

If the animal begins to experience acute cardiogenic shock as a result of the myocardial infarction we may need to administer epinephrine at a low dose (0.01 mg/kg) every 3–5 min early in resuscitation efforts; high dose (0.1 mg/kg) for repeated dosing. For perioperative hypotension we will initiate norepinephrine drip starting at 0.5mcg/kg/min and titrating as needed up to 2mcg/kg/min. Because of the nature of the transmural infarction epinephrine and norepinephrine are likely to cause increased ectopy, including possible ventricular tachycardia or ventricular fibrillation. To mitigate this risk, all epinephrine boluses will be administered with a prophylactic bolus of amiodarone (2.5mg/kg). Norepinephrine drips will be given under closely monitored conditions and amiodarone will be given if required for arrhythmias.
Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive Buprenorphine 0.01-0.02mg/kg IM.
The canine remains in the post-operative recovery cage until the following morning when they are moved to the standard chain link run with padding and blankets. They are given buprenorphine 0.01-0.02 mg/kg IM twice a day for three days for analgesia. Carprofen (2mg/kg PO) will given for 7 days after the buprenorphine regimen is completed. Famotidine 0.5-1.0 mg/kg PO or IV can be given as needed for nausea or appetite stimulant (metoclopramide 0.2-0.5 mg/kg IV or bismuth subsalicylate 262 mg PO, can be given if needed). Some canines are too active post operatively which prevents incision healing. In this case, they are given Diazepam 0.2-2mg/kg IM or PO twice a day as needed. Cefpodoxime 5mg/kg PO is given once a day for 10 days to prevent wound infection. If there is ongoing concern for wound infection while receiving Cefpodoxime (Baytril 5-20mg/kg PO can be given once a day for 10 days). IV Fluids are available with or without 5% dextrose if an animal has not resumed normal eating habits within 2 days. . Canine weights will be observed and recorded daily while on antibiotics and then twice a weekly thereafter.
They will also receive Amiodarone PO (IV is available if needed) 5mg/kg daily for 7 days post operatively.

Some subjects will be transferred to protocols # 02289 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 protocol #02289. 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. Subjects will be returned to this protocol baring any physical impairments mentioned above, after the completion of procedures on protocol #02289.

2. Terminal surgery. The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation.

Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose
A 4^{h} or 5th intercostal thoracotomy will be performed. Basic electrophysiologic studies will be performed in vivo, including measurement of effective refractory period, monophasic action potential and standard programmed stimulation protocols, including measurement of atrial effective refractory period. In order to most accurately view autonomic nerve activity during an EP study, isoflurane will be turned off for a period of about 30-45 minutes.
In order to most accurately view autonomic nerve activity during an EP study, isoflurane will be turned off for a period of about 30-45 minutes. During this time, pentobarbital will be administered as follows to keep the animal in a surgical plane of anesthesia. We will give 4-5mg/kg of pentobarbital then turn off the isoflurane. The heart rate will be monitored closely. Average heart rate for the dogs under anesthesia is 85-100bpm. If heart rate increases by more than 5bpm from the average to this point, small doses of pentobarbital (2-3 mg/kg not to exceed a total 30 mg/kg including the loading dose) will be administered to effect. Once the nerve recording is finished, isoflurane will be resumed until end of surgery. Isoflurane has a nature of suppressing nerve activity and does so more than pentobarbital. For this reason, isoflurane is removed. If the maximum dose of pentobarbital is reached during surgery, isoflurane must be resumed and the EP study completed under the effects of pentobarbital.

Blood will be collected from coronary sinus as well as aorta by direct sampling. The dog will be exsanguinated. Tissues will be collected and preserved in 4% formaldehyde for 1 hour, before being stored in 70% alcohol. Additional tissue will be snap frozen in liquid nitrogen and stored for histopathology and molecular biology.

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3.Lead revision. This is a surgical procedure to be performed only in those animals that have already undergone pacemaker implantation that happened to have a lead dislodgement of either the RA or the RV lead. This lead revision will be via a thoracotomy procedure. Procedures, surgical technique and recovery would be the same as surgery 1".

4.

Wound revision. Canines will on occasion be subject to surgical wound dehiscence. These animals will be returned to the OR for repair of the dehiscent wound, and drainage of any infectious or noninfectious fluid collections. . The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given is Brevital is unavailable) to effect and placed on a mask for isoflurane induction. Isoflurane 2-4 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. The procedure will be less than 30 minutes. The animal will recover in the post-operative recovery cage with a warming pad until they can walk to their run. They will be given Meloxicam 0.2 mg/kg IM after surgery and Cefpodoxime 5mg/kg PO for 10 days.

Personnel. Complete the table below for each individual who will be involved in any of the surgeries on this protocol.

Name	Surgery	Role in Surgery

	#(s) (see Item 1)	Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	**1,2,3,4**	**(X)**	**(X)**	**(X)**	( )
[redacted]	**1,2,3,4**	**()**	**(X)**	**( )**	( )
[redacted]	**1,2,3,4**	**(X)**	**(X)**	**( )**	( )
[redacted]	**1,2,3,4**	**X)**	**(X)**	**( )**	( )
[redacted]	**1,2,3,4**	**( )**	**(X)**	**(X)**	( )
[redacted]	**1**	**(X)**	**(X)**	**( )**	( )

Location of surgery. Complete the table below for each location where surgery on this protocol will be performed.

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space no Dedicate to Surger
[redacted]	[redacted]	**1,2,3,4**	**(X)**	( )*	( )*
			()	( )*	( )*
			( )	( )*	( )*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
►

Pre-operative protocol.

a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

Surgery #(s) (see Item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	(x) – 12 hours	( )	(x) – brachial v	( ) --
2	(x) -- 12 hours	( )	(x) -- brachial v	( ) --
3	(x) – 12 hours	( ) –	(x) – brachial v	( ) --
4	x) – 12 hours		(x) – brachial v	

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Buprenorphine	1,3,	0.01-0.02 mg/kg	IM	once	immediate
Acepromazine	1,2,3,4	0.05-0.1mg/kg	IV	Once	1 hour prior to surgery
Pentobarbital–	1,2, 3,4	Up to 30mg/kg to effect	IV	once	immediate
Brevital	1,2,3,4	6-10 mg/kg to effect	IV	Once	immediate
famotidine	1,3,4	0.22-0.44mg/lb	oral	once	immediate

Amiodarone	1	9mg/kg daily loading dose x7days, and 5mg/kg daily for 7 days	PO	Daily	14days
Penicillin	1,3	900,000unit	IM	Once	immediate

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ► **Hair to be clipped over the left thorax extending up to the neck and down to mid thorax (T7-8). Hair also to be clipped in the left flank. Surgery sites prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.**

Surgery 2 ► **Hair to be clipped over the left thorax extending up to the neck and down to mid thorax (T7-8). Hair also to be clipped in the left flank. Surgery sites prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.**

Surgery 3 ►**.**
Hair to be clipped over the left thorax extending up to the neck and down to mid thorax (T7-8). Hair also to be clipped in the left flank. Surgery sites prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.

Surgery 4
Hair to be clipped over the left thorax extending up to the neck and down to mid thorax (T7-8). Hair also to be clipped in the left flank. Surgery sites prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.

d. Intra-operative medications. Complete the table below for each agent that will be administered to the animal during surgery.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Isoflurane –	No	1,2,3,4	2-4%	Inhalation	Continuous
Pentobarbital	No	1,2,3	Up to 30mg/kg to effect	IV	Once before surgery if brevital not available and during surgery for non survival

Brevital	No	1,2, 3,4	6-10 mg/kg to effect	IV	Once
Epinephrine	No	1,2	Low dose (0.01 mg/kg) every 3–5 min early; high dose (0.1 mg/kg) after prolonged; 1 ml	IV	If needing for hypotension: Low dose: Every 3-5 mins High Dose: After prolonged VF (15 mins)
Levophed (norepinephrine)	No	1,2	IV drip initiated at 0.5mcg/kg/min with titration up to 2mcg/kg/min or higher	IV	If needed for hypotension: continuous
Lidocaine	No	1,3	1-2mg/kg bolus, followed by 1-2mg/kg/hr infusion	IV	Bolus administration with repeat boluses as needed, Additionally, an hourly infusion can be initiated and continued intraop until bag is completed after surgery has been completed.
Amiodarone	No	1,3	7mg/kg bolus, repeated (2.5-5mg/kg) every 30 minutes as necessary	IV	Bolus administration, repeated every 30 minutes as necessary

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.

e. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals <u>during</u> surgery (e.g., warming, cushioning, etc.).

The dog will be placed on a warming pad, and blankets placed over extremities and lower half of body during surgery. Warmed IV normal saline will be administered for maintenance IV fluids. Animals will be placed on a water heated pad during the protocols and body temperatures will be monitored. All animals will have venous access through which fluids can be given to prevent dehydration. Animal will continue with water heated pads during the immediate postsurgical period.

f. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.

The level of anesthesia will be continuously monitored by observation of arterial pressure, heart rate, and oxygen saturations every 15 minutes throughout surgery. Any increase in pressure or heart rate in response to surgical stimuli will be interpreted as indicative of inadequate anesthesia and supplemental doses will be given. In addition, corneal, palpebral, and toe-pinch responses will be monitored every 15 minutes and supplemental anesthesia given as needed.
In order to most accurately view autonomic nerve activity during an EP studies isoflurane must be turned off for a period of 30-45 minutes. During this time Pentobarbital (5mg/kg) is given as follows to keep the animal in a surgical plane of anesthesia. The heart rate will be monitored closely. Average heart rate for the dogs under anesthesia is 85-100bpm. If heart rate increases by more than 5bpm from the average to this point, small doses of pentobarbital (2mg/kg) will be administered to effect. Each dog will be weighed before surgery to determine the maximum dose of pentobarbital (30mg/kg) that is allowed to prevent them from overdose. Once the nerve recording is finished, isoflurane will be resumed until end of surgery. Isoflurane has a nature of suppressing nerve activity and does so more than pentobarbital. For this reason, isoflurane is removed. If the maximum dose of pentobarbital is reached during surgery, isoflurane must be resumed and the EP study completed under the effects of isoflurane.

8. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery # (see Item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other *
1	16-18 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )
3	16-14 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )
4	12-14 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	

* Describe any "other" measures to be taken to maintain sterility during surgery.
►

b. For each surgery, describe the immediate post-operative support to be provided to the animals.
Surgeries 1, 2, 3, 4
Animal will be place in a recovery cage with a warming pad and covered in blankets. They will be visually monitored for heart rate and respiration until sternal.

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1, 3,4	Buprenorphine	0.01-0.02 mg/kg	IM	Q8-12 hrs	1-3 days
1, 3,4	Carprofen	2 mg/kg	Oral	SID	7 days
1, 3,4	Diazepam	0.2-0.4 mg/kg	Oral or IM	BID if needed	PRN
1, 3,4	meloxicam	0.2mg/kg	IM or SQ	As an alternative to Carprofen	up to 7 days

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
►

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1, 3,4	Baytril	5-20mg/kg	Oral	Daily, as needed for wound infection	10 days

1, 3,4	Cefpodoxime	5mg/kg	Oral	Daily, as needed for wound infection	10 days
1,3,4	bismuth subsalicylate	262mg	oral	SID	As needed
1,3, 4	famotidine	0.5-1.0mg/kg	oral	SID	As needed
1,3, 4	Metoclopramide	0.5 mg/kg	IV	SID	As needed

e. Post-operative monitoring. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

i. Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Continuously	At least 4-6 hours post op	[redacted]
3	Continuously	until sternal	[redacted],
4	Continuously	Until sternal	[redacted]

ii. Post-operative monitoring after the immediate post-operative period

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Hourly between 8 am-4:30 PM	3-4 days	[redacted]

3	Every 6-12 hours	2-3 days	[redacted],
4	Every 6-12 hours	2-3 days	[redacted]

f. Post-operative consequences and complications.

i. For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1 ► During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by exsanguination under anesthesia. Rib fractures may occur during the thoracotomy. If this occurs the veterinarian will be notified as analgesics may need to be given for a longer duration.

Pneumothorax (inadequate seal of thoracotomy incision) can also occur immediately post operatively. This will require an emergency surgery to reclose the incision. Animal may go in to cardiogenic shock during or after the procedure. If hypotensive, they will be provided with amiodarone and epinephrine to increase blood pressure. Sudden cardiac death is a risk during this procedure. Amiodarone and epinephrine will be available. Animal will be monitored for arrhythmias and tachycardia pacing or defibrillation can be employed as well as treatment with amiodarone or lidocaine.. MI is an expected outcome of this procedure and all efforts will be made to ensure it is survivable but if the infarct is too large it may be unrecoverable. If pulmonary fusion occurs, during surgery, due to the close proximity to the lungs the animal will be monitored hourly for 24 hours. Blood pressure and pulse oximetry will be recorded hourly. If the animal shows any signs of respiratory distress and vitals decline, the animal will be euthanized via a fatal pentobarbital injection

-Post operatively, the major concern is pain and this will be treated with analgesics. Standard wound care will be provided. Animals that are slow to resume normal eating patterns will be given fluids either IV or SC and given anti-emetics.

Surgery 2►N/A

Surgery 3► During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by exsanguination under anesthesia. Pneumothorax (inadequate seal of thoracotomy incision) can also occur immediately post operatively. This will require an emergency surgery to reclose the incision. Hypertension is a consequence of this surgery and analgesics will be provided.

Post operatively, the major concern is pain and this will be treated with analgesics. Standard wound care will be provided. Animals that are slow to resume normal eating patterns will be given fluids either IV or SC and given anti emetics.

Surgery 4►

Post operative infection or intraoperative bleeding are the main concerns. If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by pentobarbital overdose. Post operatively, the major concern is pain which can be treated effectively with analgesics. Animals who remain anorexic or with reduced appetite will be given IV or SQ fluids and treated with anti emetics (metoclopramide and famotidine**)**

ii. List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1 ► Major nonsurvivable postoperative complications are described above. Any animal in pain or distress that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain will be suspected in the presence of weight loss (10% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression. Wound infection not cured by antibiotics, pneumothorax, respiratory distress symptoms, and hypertension. In addition, evidence of symptomatic heart failure that is not resolved with low sodium diet, or diuresis within 1-2 weeks of diagnosis will be grounds for euthanasia.

Surgery 2-NA

Surgery 3, Major nonsurvivable postoperative complications as described above. Any animal in pain or distress that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of weight loss (10% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression. Wound infection not cured by antibiotics, pneumothorax, respiratory distress symptoms, and hypertension.
Surgery 4 -- The major concern for this minor procedure is pain and this will be treated with analgesics. Standard wound care will be provided. Animals who are slow to resume normal eating patterns will be given fluids either IV or SC and anti-emetics..

iii. In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)
►none.

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	[redacted]	[redacted],	(X)	( )
2	[redacted]	[redacted]	(X)	( )
3	[redacted]	[redacted]	[redacted]X)	( )
4	[redacted]	[redacted]	X	

9. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
Number	Brief Description	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	Irradiation	Imaging	Other**
1	**Radio Telemetry. Cage cleaning and letting of dogs out of cage will need to be done one dog at a time, so as to avoid data crosstalk between cages (receivers meant for another dog picking up data from this dog as the dog runs around the room). Also, times will have to be pre-specified so the P.I. can expect when data drop out or potential cross talk occurs.**	**(X)**	**( )**	**( )**	**( )**	**( )**	**( )**	**( )**	**( )**
2	**Drug Challenge**	(X)	( )	( )	( )	( )	( )	( )	( )
3	**Holter monitor**	x)	( )	( )	()	( )	( )	( )	( )

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.
►

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure 1 ► Typically cage cleaning is performed daily with dogs let out of the cage in random fashion. However, because the receivers installed in each cage has the capability of picking up signals from any dog that is in close proximity to it, letting dogs out all at the same time has the ability to cause data cross talk, thus invalidating the data for that period of time when the dogs are out of the cage. For this reason, the dogs with transmitters in the same room, have to be let out individually, returned to the cage, before another dog with a transmitter be let out of its cage. Cleaning times will be posted clearly on the dog runs and communicated to the VMU supervisor and caretakers.

Special Procedure 2 ► Drug Challenge. In order to understand and validate recordings between stellate and vagal nerve, we plan to pharmacologically stimulate autonomic nerves by administration of short-acting intravenous vasoactive drugs (clonidine or phenylephrine) during the chronic monitoring phase.

These drugs used are ones commonly used in clinical practice in humans but have been also used in canines. They are all short-acting drugs whose half-lives do not exceed 12 hours when given orally. Therefore, when administered IV, their effects peak within minutes and half-lives usually less than 4 hours as described below:

1. IV clonidine (10 µg/kg) is an alfa-2 agonist which will suppress central sympathetic nerve discharge by acting on pre-synaptic alfa-2 receptors in sympathetic nerve terminals. IV Clonidine peaks within an hour and has a plasma half-life of 2-3 hours. Blood pressure and heart rate are expected to drop but the doses used have been reported in the literature [Cavero, Br. J Pharmacol 1980; 70:269]. We expect that the effects are transient and will not have long term sequelae.
2. IV phenylephrine 0.1mg. Phenylephrine is a vasopressor used to increase BP. This will be given as an IV bolus. The increase in BP will suppress sympathetic nerve activity and potentiate vagal nerve activity. We expect that the effects are transient and will not have long term sequelae. [Varma S, Circulation Research 1960;8:1182].

The animals will be challenged twice with each of the above drugs. Once approximately 4 weeks following recovery from survival surgery. The second time will be after induction of PVC-related cardiomyopathy (>3 months later). The drugs will be challenged one at a time on a separate day each. See section b.III. below for details on procedure.
We will *maintain a log* of blood pressure readings, heart rate, time of administration and physical characteristics during the monitoring phase and keep this information in the animals folder for review.

Special Procedure 3 ►
Holter. Holter monitor will be worn for 24-48 hours. As in humans, this procedure is not painful and will not cause any distress to the animal. We do not expect to require any type of restraint to set up the holter monitor. After the required time is met, Holter will be removed and sent for analysis of PVC burden or percentage, and autonomic activity Normal husbandry can be conducted but the staff should not try to remove the holter and notify the research staff if the dog has chewed the outer covering or wires.

b. Explain why each of these special procedures is necessary:

Special Procedure 1 ► As soon as PVC software patch is enabled, DSI radiotelemetry device will be turned ON to record vagal and renal autonomic nerve activity and monitor the relationship between cardiac autonomic nerve activity and the development of PVC-induced CM.

Special Procedure 2 ► The purpose of the IV pharmacological challenge is to characterize the behavior of the renal sympathetic nerve recordings and its correlation with cardiac sympathovagal nerves at baseline and after PVC-induced CM has developed. The interventions are designed to perturb blood pressure (in the case of adenosine, hydralazine, nitroglycerin and clonidine) and consequently baroreflexes, which will stimulate or suppress cardiac sympathovagal and/or renal sympathetic nerves, or suppress central sympathetic outflow (in the case of clonidine) or suppress the peripheral effects of vagal nerves (in the case of atropine).

Special procedure 3 ►
Cardiac Holter monitor will be obtained in order to corroborate the target or programmed PVC burden [redacted]) as per protocol.

Special procedure 4 ►

Personnel. Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

Procedure Number (see Item 1)	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	[redacted]	[redacted]
2	[redacted]	[redacted]
3	[redacted]	[redacted]
4		
5		
6		
7		

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number (see Item 1)	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1	X		( )[a]	( )[b]
2		IV drugs administered during this challenge can cause dizziness, loss of consciousness, high blood pressure, racing heart, anxiousness, and flushing sensation. All effects are short lived.	(X)[a]	( )[b]
3	x		(X)[a]	( )[b]

a. For each procedure for which potential pain and/or distress is expected, but <u>WILL be prevented or alleviated</u> by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
2	Acepromazine	0.05-0.1mg/kg	IV and oral	once	0
3	furosemide	4-10mg (or 0.2-0.5mg/kg)	IV and PO	daily or BID	1-14

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

b. For each procedure for which potential pain and/or distress is expected and will NOT be prevented or alleviated, provide the scientific justification for this:

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number (see Item 1)	Monitoring Methods	Endpoint Criteria
1	2 DSI telemetry devices are attached to dog runs to monitor routine, daily behavior	these monitors will not cause pain or distress.
2	Present with dog and blood pressure monitoring during procedure and close observation throughout full recovery. Also Pacemaker ICD devices will be continuously interrogated during this procedure for realtime cardiac electrical activity monitoring. The animal will be observed for any signs of exercise intolerance, heavy breathing, loss of consciousness and coughing. Research personnel will monitor the animal's DSI recording for 60 minutes post drug administration.	Drug challenge would not be obtained in those animals with any kind of distress / recent wound or surgical procedures.

3	Animals will have Holter monitors placed by team in the morning of the day to be recorded, and they will be returned to cages for the remainder of the day. Prior to departing for the day the team will reassess Holter monitor placement and make sure that the computer is accurately recording the data transmitted from the Holter device	This procedure will be complete at the end of 24 or 48 hours, or if the essential information cannot be obtained.

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Richmond, VA - 652	Other - pending	Effect of chronic premature ventricular contractions on the remodeled ischemic heart

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)	This ACORP uses a canine model of premature ventricular contractions (PVCs) and myocardial infarction and seeks to determine how PVCs contribute to myocardial dysfunction and disease. This protocol is technically challenging due to the creation ischemic cardiomyopathy being essential to the research study. The investigator is commended for the pretreatment of the dogs with amiodarone to prevent secondary arrhythmias, a clear rationale for all procedures performed and drugs administered, the comprehensive nursing care plan, the detailed humane endpoints, the treadmill (reward based) training program that allows each dog to learn at their own pace, an experienced research team, and the sound justification for the use of a canine model. 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	In item C.2.b, the investigator states "Prior work in our PVC induced cardiomyopathy model has shown that there is an absolute reduction of 20% of LVEF (60%-40%) over the 12 week period." LVEF is presumed to refer to is left ventricular ejection fraction; please clarify and explain the significance of LVEF. In item C.2.c, the investigator describes the dogs will "…undergo a single cardiac Holter monitor… " but later states "We plan to repeat these Holter monitors up to twice weekly throughout the testing period in order to correlate this data with the implanted DSI device." Please clarify.

(cont.)

	Also in item C.2.c, the investigator refers to classes of heart failure (class 1 HF, class 2 HF, etc.) without (in all cases) clearly defining the clinical signs of each one; it would be helpful to provide a description of each heart failure class.
Item M	In item M, the investigator notes that animals that display clear signs of heart failure will be transitioned to a low sodium diet, please describe the diet and the transition plan in Appendix 6.
Item U	If the euthanasia method is an overdose of pentobarbital, please clarify how death is confirmed or if a second physical method of euthanasia is performed.
Appendix 5	Item 1.a and 1.b of this appendix, the investigator states "Wound revision - it is not uncommon for surgical wound dehiscence to occur immediately after surgery."and Wound revision- This would occur 2-7 days after the initial surgery if needed." Wound dehiscence soon after surgery is often related to the sutures being tied too tightly, which compromises blood supply. In dogs, the sutures should be tightened (tied) just enough to appose the skin edges; the surgeon should be able to place the tip of a small hemostat beneath each interrupted suture. Skin breakdown over a subcutaneously implanted device is also usually related to pressure necrosis. The information found at the following link may be helpful: https://www.acvs.org/files/proceedings/2012/data/papers/122.pdf

Appendix - Additional Suggestions for Improvement

Comment 1: <u>Part B.</u> This section could be made clearer by mentioning the main focus of the study at the beginning. Also, the specific relevance to Veterans' health is not specified. Try putting something like this right after the first sentence:

Unfortunately, many patients who survive a heart attack go on to develop a condition called chronic ischemic heart disease in which the remaining heart tissue does not get enough blood flow for it to function properly. The goal of this study is to understand why this condition develops after a heart attack so ways to prevent it can be developed. This is particularly relevant for Veterans who were exposed to Agent Orange, which the VA recognizes as linked to ischemic heart disease (see https://www.publichealth.va.gov/exposures/agentorange/conditions/ischemicheartdisease.asp accessed 12/18/17)

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

Canines are used because they have very similar physiology 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 and leads available for this kind of study are too large for small species such as rabbits, rats, or mice. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated (approximately 2 inches long, ¼ inch thick and 1.5 inch wide) electronic defibrillator / pacemaker that was specifically developed for this work. The radiotelemetry device we need to implant is also too large for smaller species. There are

(cont.)

biological pacemakers that have been developed in smaller, less sentient species, but they cannot store and analyze data and are difficult to reprogram.

Comment 3: <u>Part H:</u> Since size is one of the justifications for using dogs in this study, it would be appropriate to specify the breed of dog here.

Comment 4 <u>Part W1 table:</u> The searches in the original table will only bring up a paper that include <u>all</u> of the search terms listed. This will eliminate many important papers that only have some of the search terms. 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.

   ► ( ) **N/A**
   ► (X) **Painful or distressing procedures:**

   ► chronic PVC-induced left ventricular dysfunction
   myocardial infarction by ligating a coronary artery
   thoracotomy
   symptomatic congestive heart failure

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 <u>Animal Use Alternatives</u> as the main topic)	**12-18-2017**	**2000-2017**	**N/A – this search was for alternatives to using animals.**	**chronic PVC-induced left ventricular dysfunction**	X	X	X	
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-18-2017**	**2000-2017**	**N/A – this search was for other species and techniques that could be used.**	**chronic PVC-induced left ventricular dysfunction**	X	X	X	
PubMed (limit set	**8-26-**	**1966-2017**	**N/A – this search was for**	**PVC-induced left ventricular**	X			

(cont.)

for "other animals")	**2014**		**other species that could be used.**	**dysfunction**				
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-19-2017**	**2000-2017**	**chronic PVC-induced left ventricular dysfunction**	**chronic PVC-induced left ventricular dysfunction**	X	X	X	
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-18-2017**	**2000-2017**	**Myocardial infarction**	**myocardial infarction, coronary artery, canine**	X	X	X	
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-1-2017**	**2000-2017**	**thoracotomy**	**thoracotomy, "cardiac surgery"**	X	X	X	
ALTBIB (Search PubMed using ALTBIB animal alternatives search strategy)	**12-18-2017**	**2000-2017**	**symptomatic congestive heart failure**	**"symptomatic congestive heart failure", canine**	X	X	X	
PubMed	**12-19-2017**	**1966-2017**	**N/A**	**Premature ventricular contractions, left ventricular remodeling, cardiomyopathy**				X

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:

This study focuses on chronic PVC-induced left ventricular dysfunction, and our ALTBIB search for alternatives to using animals for studying this yielded no papers at all. There are no computer models or in vitro models for this work.

We also ran an ALTBIB search for other animal models or methods to study PVC-induced left ventricular dysfunction, and again no papers came up.

In order to bring up as many papers as possible we ran a regular PubMed search for PVC-induced left ventricular dysfunction with the search limited to animals. That brought up five papers, four on dogs and one on pigs. There are no smaller mammal or non-mammalian models available for this research.

Comment 6: <u>part W3 (statistics):</u> This section would be strengthened by adding a sentence at the end that directs the reader to the detailed statistical analysis earlier in the ACORP:

(cont.)

Please see section C2b for details.

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

We ran multiple searches for additional refinements:

1) A search on ALTBIB for “chronic PVC-induced left ventricular dysfunction” yielded no papers.
2) A search on ALTBIB for “myocardial infarction, coronary artery, canine” yielded 28 papers, none of which were refinements over our current techniques.
3) A search on ALTBIB search for “thoracotomy, cardiac surgery" gave 5 papers. Two were on rats, one was one sheep, and one was on mice. The fifth paper was about transcatheter pulmonary valve replacement, which is very different from what we are doing in this study. We did not find refinements for thoracotomy in canines for our studies.
4) A search on ALTBIB for “symptomatic congestive heart failure, canine” yielded no papers.

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 how a heart attack leads to premature ventricular contractions that cause left ventricular remodeling and eventual ischemic cardiomyopathy in a situation where there are no drugs or mutations that could affect the process. Our PubMed search for “Premature ventricular contractions, left ventricular remodeling, cardiomyopathy” brought up 22 papers. Twenty-one of the papers were not closely related to this project. Five of these papers were observational studies looking at characteristics of groups of heart patients, three were case studies on individual patients, three were on drug effects, three were studies on how various mutations affect the development of heart disease, and two were on ablation as a treatment. There were single papers on renal denervation, stem cell transplants, a new imaging method, cardiac neuronal control, and non-ischemic cardiomyopathy. The remaining paper is from our laboratory but does not actually look at how a heart-attack leads to the eventual development of ischemic cardiomyopathy, which is the focus of this study. The work we are doing in this study is unique and has not been done before.

(cont.)

Literature search 652 Richmond [redacted]

1) How is this research relevant to Veterans health?

Unfortunately, many patients who survive a heart attack go on to develop a condition called chronic ischemic heart disease in which the remaining heart tissue does not get enough blood flow for it to function properly. The goal of this study is to understand why this condition develops after a heart attack so ways to prevent it can be developed. This is particularly relevant for Veterans who were exposed to Agent Orange, which the VA recognizes as linked to ischemic heart disease (see https://www.publichealth.va.gov/exposures/agentorange/conditions/ischemicheartdisease.asp accessed 3/11/18)

2) Is this work <u>unnecessarily</u> 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 (set to best match)	3/11/18	All available years	Premature ventricular contractions, left ventricular remodeling, cardiomyopathy	29

This study will focus on how a heart attack leads to premature ventricular contractions that cause left ventricular remodeling and eventual ischemic cardiomyopathy in a situation where there are no drugs or mutations that could affect the process. A PubMed search for “Premature ventricular contractions, left ventricular remodeling, cardiomyopathy” brought up 29 papers.

Twenty-eight of the papers were not closely related to this project. Eight of these papers were observational studies looking at characteristics of groups of heart patients, three were case studies on individual patients, and seven were studies on how various mutations affect the development of heart disease. There were single papers on renal denervation, stem cell transplants, a new imaging method, doxorubicin-induced cardiomyopathy, cardiac neuronal control, a drug study in rats, and a review article. There were also three clinical trials (an LVAD study, a defibrillator study, and a drug study). None of these papers focused on how a heart attack leads to premature ventricular contractions that cause left ventricular remodeling and eventual ischemic cardiomyopathy.

The remaining paper is from this laboratory but does not actually look at how a heart-attack leads to the eventual development of ischemic cardiomyopathy, which is the focus of this study. The work we are doing in this study is unique and has not been done before.

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	3/11/18	All available years	Premature ventricular contractions, left ventricular remodeling, cardiomyopathy	0

This study focuses on chronic PVC-induced left ventricular dysfunction, and an ALTBIB search for "alternatives to using animals" for studying this yielded no papers at all. There are no computer models or in vitro models for this work.

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

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 animal alternatives search strategy - all citations	3/11/18	2000-present	Premature ventricular contractions, left ventricular remodeling, cardiomyopathy	2
PubMed (set for best match and "other animals")	3/11/18	All available years	Premature ventricular contractions, left ventricular remodeling, cardiomyopathy	10

An ALTBIB search for alternatives brought up only two papers. Both were on particular genetic defects, which is not what this study is about.

In order to bring up as many papers as possible a regular PubMed search was run with the search limited to animals. That brought up 10 papers, of which seven were on small animals (mice, rats, rabbits, and cats) Unfortunately, the electronic pacemakers and leads available for this kind of study are too large for these species. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated (approximately 2 inches long, ¼ inch thick and 1.5 inch wide) electronic defibrillator / pacemaker that was specifically developed for this work. The radiotelemetry device they need to implant is also too large for smaller species. There are biological pacemakers that have been developed in smaller, less sentient species, but they cannot store and analyze data and are difficult to reprogram.

Of the remaining three papers, two were on pigs and one on dogs.

Unfortunately, the electrophysiology of the pig heart differs from the human and canine heart in a significant way, specifically the endocardium and epicardium are activated simultaneously in the swine heart but not in the human or canine heart [Lelovas 2014], and this discordance in the human and dog heart can play a role in the development of cardiomyopathy. Additionally, dogs have a His-Purkinje system located in endocardium, very similar to the human heart, that pigs and other larger animals do not have [Newton 2004].

Dogs are used 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)?**

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	How many papers were found?
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	chronic PVC-induced left ventricular dysfunction	chronic PVC-induced left ventricular dysfunction	0
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	Myocardial infarction	myocardial infarction, coronary artery, canine	32
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	thoracotomy	thoracotomy, "cardiac surgery"	5
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	symptomatic congestive heart failure	"symptomatic congestive	0

				heart failure", canine	

We ran multiple searches for better methods:

1) A search on ALTBIB for “chronic PVC-induced left ventricular dysfunction” yielded no papers.
2) A search on ALTBIB for “myocardial infarction, coronary artery, canine” yielded 32 papers, none of which were refinements over our current techniques.
3) A search on ALTBIB search for “thoracotomy, cardiac surgery" gave 5 papers. Two were on rats, one was one sheep, and one was on mice. The fifth paper was about transcatheter pulmonary valve replacement, which is very different from what we are doing in this study. We did not find refinements for thoracotomy in canines for our studies.
4) A search on ALTBIB for “symptomatic congestive heart failure, canine” yielded no papers.

This group has extensive experience in this surgical model of PVC-induced cardiomyopathy, a model that they designed. The method has been refined so the cardiomyopathy develops gradually without symptomatic congestive heart failure or signs of distress.

<u>The treadmill procedure is designed to minimize distress for the dogs:</u>

Dogs allowed to them explore the exercise room and equipment until they have become comfortable with those surroundings. Presence of normal, relaxed behavior will signal that the dogs are ready for the next step, which is putting them on the treadmill while it’s turned off. This will occur in small steps, putting them on for seconds and then extending the time. Each positive reaction will be rewarded with treats to encourage the dogs’ learning process. When the dogs have become relaxed with the task of being on the still treadmill, they will next be put on the treadmill at its slowest speed, 0.5 mph. Two people will assist in this process; one person will hold the leash of the dog and stand in front of the treadmill offering rewards for positive behavior while the other will stand behind the animal making sure that it does not slide off of the machine or jump off of the sides. This person will also to help the dog move its feet until it begins to understand and be comfortable with the movement. The process will take as long as needed to have the dogs become comfortable with the treadmill**.**

The treadmill workout will be done a total of 4 times in our study. The first 2 workouts will be performed 1-2 days apart at baseline about 2 weeks post-surgery after sutures have been removed. The final 2 treadmills will occur 1-2 days apart at the end of the study before final surgery.

Each workout lasts 10 minutes, in which the dogs will complete 3 stages, each lasting 3

minutes. at the first stage is at 1.1 mph followed by three minutes at 2.3 mph, and then three minutes at 3.3 mph. (Normal human walking speed is about 3 mph).

The procedures for echocardiograms, blood draws, etc., are also designed to minimize distress for the dogs:

Non-surgical procedures such as echocardiograms, electrocardiography, pacemaker interrogation and blood draws will be performed in conscious dogs with minimal or no sedation. In order to achieve this, all animals will undergo training to lay or sit down still for 20-30 minutes during the procedures. We estimate that this training will take from 2-4 weeks. Methods used for training will consist mostly of repetition with rewards as the periods of lying or sitting still are gradually extended.

However, if an animal cannot be trained to sit or lie supine for 10 minutes for the echocardiogram, we will first attempt to mildly sedate the animal with Acepromazine (0.05-0.1mg/kg) given PO approximately 1 hour prior to the procedure. If this is unsuccessful we will have to perform echocardiogram under general anesthesia with endotracheal intubation. We will use Brevital (6-10mg/kg) IV to effect (or Pentobarbital 30mg/kg, if Brevital is not available). Animals will be intubated, mechanically ventilated and anesthetized with isoflurane 1-3%. After the echocardiogram, they will be allowed to recover from anesthesia in a post-operative recovery cage until able to walk to their kennel. No analgesics will be necessary due to the non-invasive nature of this procedure.