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► **Autonomic Nerve Activity and Cardiac Arrhythmias**

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

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

►( ) Department of Veterans Affairs.
►( ) US Public Health Service (e.g. NIH).
►(V) Private or Charitable Foundation -- Identify the Foundation: American heart Association
►() University Intramural Funds – Identify the University and Funding Component:
►( ) Private Company – Identify the Company:
►( ) Other – Identify Other Source(s):

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
      ►The predecessor protocol encompassed studies examining the role of autonomic nerve activity in several different arrhythmia models. These included (a) PVC-induced cardiomyopathy model, (b) Atrial fibrillation model, (c) Heart failure model, and (d) myocardial infarction model. In the past 3 years as we established the autonomics laboratory, we have focused our attention on the PVC-induced cardiomyopathy model. We have established the following:
         (i) Baseline studies examining the acute and chronic effects of PVC-induced cardiomyopathy on autonomic nerve activity.
         (ii) Recovery studies: examining what happens to autonomic nerve activity as PVCs are disabled and the PVC-induced cardiomyopathy resolves.

(iii) Denervation studies: we are in the midst of examining the effects of afferent denervation on altering the development of or recovery from PVC-induced cardiomyopathy.

In this renewal, we are removing all other arrhythmia models and focusing only on the PVC-induced cardiomyopathy group. We are proposing new studies on efferent denervation which will be a fourth group to be performed on the PVC-induced cardiomyopathy group.

We wish to emphasize that the intervention arms of this protocol, i.e., afferent AND efferent denervation, are pilot experiments that have never been performed in chronic PVC dogs models prior to this.

(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
► For studies (i) to (iii), we used 7 animals for study (i), 5 animals for study (ii), and 8 animals for study (iii). We would also like to clarify that for studies (i), they were animals that were technically part of [redacted] protocol. We added DSI nerve monitoring to [redacted] PVC-induced cardiomyopathy protocol. Thus, these animals were jointly covered by both [redacted] 's and the present protocol.

(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.
►We had 3 animal fatalities for studies (iii) . Two of them were specifically related to the afferent denervation procedure, and these animals experienced intractable nausea due to gastroparesis from mechanical bilateral vagal injury. We modified the protocol by performing staged unilateral denervation, thus adding a third survival surgery, and by minimizing mechanical manipulation of the vagus trunk. Using this approach, both pilot animals that have undergone bilateral staged denervation have done well. They have experienced some transient nausea and vomitting and limited weight loss in the first week after procedure, but these have resolved with metoclopramide, a prokinetic agent, and supplemental nutrition. The animals also have a normal appetite and are able to regain the lost weight with supplemental nutrition. With adequate treatment, they do not appear to be in overt distress and these side effects appear to eventually resolve.

The 3rd animal fatality was related to ventricular fibrillation induced by active fixation of pacing lead. For reasons unclear, the animal was not resuscitable due to recurrent VF. This is a known complication of lead implantation. To be better prepared for this, we will have on standby resuscitation and antiarrhythmic medications, including epinephrine and amiodarone.

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.

Premature ventricular contractions (PVCs) affect 1-4% of the population without structural heart disease. It is an increasingly recognized cause of damage to the heart muscle that is not associated with interruptions to the heart's blood supply. In a large multicenter study of 245 patients seen for PVC's, 67% of patients experienced a decline in outbound blood pumped from the heart with each heartbeat. This suggests that the prevalence of PVC-induced heart muscle disease (cardiomyopathy), is much higher than previously suspected. Furthermore, patients with preexisting structural heart disease can experience further declines in the output of blood pumped from the heart with frequent PVCs. Although radiofrequency ablation (the destruction of autonomic nervous system nerves- the peripheral nervous system that supplies smooth muscles and glands that are not subject to voluntary control), or anti-arrhythmic medications can suppress PVCs and reverse cardiomyopathy, ablation failure rates of 60% and PVC recurrence rates of 10-20% have been reported. Medications which treat heart irregularities may be limited by side effects and new or more frequent occurrence of pre-existing irregularities. Thus, an improved understanding of the mechanisms underlying PVC-induced cardiomyopathy is needed to determine new therapeutic strategies. This proposal will investigate problems with the autonomic nervous system (the nerves that control involuntary bodily function) in relation to the development of the disease and frequent occurrence of pre-existing arrhythmias as a result of PVC-induced cardiomyopathy in canines and evaluate the role of autonomic nerve loss (denervation) as a mechanistic and therapeutic strategy.
The proposal aims to test whether deranged autonomic nerve activity triggered by PVCs, promote the development of cardiomyopathy, and whether abnormal autonomic nerve activity further promotes the development of other arrhythmias in a heart which is rendered abnormal by PVCs. The strategy employs the ligation or ablation of nerves in the afferent limb vs the efferent limb of the autonomic nervous system network. This system consist of nerves from the heart and blood vessels (afferent limb) that travel to the central nervous system (CNS) and nerves that return to the heart from the CNS (efferent limb). By selectively targeting each of these limbs we can determine how the autonomic nervous system interacts with a normal and abnormal heart. This also potentially represents a new therapeutic strategy for PVC-induced cardiomyopathy.

C. **Experimental Design.**

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

The proposed experiments will utilize a canine model to investigate the relationship between the nerves that innervate the heart and the kidney and the development of heart rhythm disturbances (arrhythmias) and heart failure.
During an initial surgery, canines will have pacemakers and radiofrequency recording devices surgically implanted. We will implant a Data Sciences International (DSI) radiotelemetry device subcutaneously in canines to record nervous activity. This device has three bipolar channels. We will implant one channel to record the left stellate ganglion nerve activity and one channel to record cardiac vagal nerve activity and heart rhythms [Electrocardiogram (ECG)]. The third channel will be for reserved heart rhythm or blood pressure. The pacemaker will be used to create arrhythmias (PVCs) that promote the development of cardiomyopathy.

Following this initial surgery, we will initiate PVCs over a period of 12 weeks to induce a cardiomyopathy. Using the DSI recording and echocardiograms, we will quantify and characterize the changes to autonomic nerve activity and study the role of autonomic nerve loss in the development of PVC-induced cardiomyopathy and the potential benefit of modulating the autonomic nervous activity in these conditions.

We will utilize the novel technique of chronic ambulatory measurements of cardiac sympathetic and vagal nerve activity in a novel canine model of PVC-induced CM, to test the following hypotheses:
1. PVCs disrupt autonomic nervous activity (ANA) via sino-aortic baroreceptors (sensitive to changes in pressure) and/or cardiac stretch mechanoreceptors (receptors sensitive to mechanical stimuli).
2. Autonomic dysregulation is pro-arrhythmic and contributes to reduced functional capacity in PVC-induced CM. (Aim 2)
3. Autonomic denervation is anti-arrhythmic and can prevent or retard the development of PVC-CM.

This study will evaluate the reduction in cardiac contractility (intrinsic ability of the heart to contract) after frequent premature heart beats (PVC-induced cardiomyopathy) and the associated changes in autonomic nerve activity that accompanies PVC-induced cardiomyopathy. Frequent PVCs will be replicated in canines for 12 weeks (PVC period) by the implanted pacemaker placed during the first survival surgery. PVCs induce a change in autonomic nerve activity by activating baroreceptors in the major vessels, which will in turn feedback via neural pathways into the brain stem (afferent) where autonomic output originates. We plan to selectively eliminate the neural pathways that control this autonomic nerve traffic, with the goal that PVCs no longer induce a change in nerve activity as the information from baroreceptors (triggered by PVCs) no longer reaches the brainstem centers. Canines will be divided into two groups; those that undergo interruption of the autonomic nervous activity through destruction (ablation) of the nervous tissue [Denervated group] and those that retain intact autonomic nervous activity [non-denervated group]. In the denervated group, animals will be sub divided into either a sino-aortic afferent (neural pathways going towards the brain) or thoracic efferent (neural pathways going away from the brain) denervation group. The sino-aortic afferent denervation group will undergo 2 additional surgeries to complete bilateral denervation, while the Thoracic efferent denervation procedure can be completed during the initial surgery. Once the animal has recovered, they will undergo acute studies which involve a series of challenges (ventricular (PVC), atrial (PAC), Drug challenge, and exercise challenge) and baseline recordings (Autonomic nerve data acquisition, echocardiographic evaluation). Challenges will be repeated at weeks 4, 8, and 12. After the PVC period, animals will undergo a recovery period and challenges will be repeated once more. A final terminal surgery will be performed to determine the induction of heart

irregularities. Animals will be euthanized during this surgery and tissues will be collected for biochemical studies.

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 protocol will utilize the novel technique of long term ambulatory measurements of cardiac sympathetic and vagal nerve activity in the canine model of PVC-induced CM.

This study will investigate 34 animals in total over a period of 36 months. There will be 10 control animals with PVC-induced CM alone. 24 animals with undergo denervation, 8 for sino-afferent denervation and 16 animals with Thoracic Efferent Denervation (8 for sympathetic denervation alone, and 8 for sympathovagal denervation).

PVC-induced CM alone (Control) Total= 10 animals

Denervated Total= 24 animals
Sino-aortic Afferent denervated= 8 animals
Thoracic Efferent denervated Left stellate ganglion (LSG) only= 8 animals
Thoracic Efferent denervated LSG and vagal nerve= 8 animals

Total animals= 34

All animals will undergo an initial surgery to implant pacemakers (ventricle and atrial) and a radiofrequency device (DSI). This surgery will be performed via left thoracotomy. The animals will be allowed 14 to 21 days of recovery before starting acute studies.

The afferent denervated group will require two additional surgeries in order to complete bilateral sino-aortic denervation, while the efferent group (LSG only or LSG and vagal nerve) procedure can be completed during the initial surgery.

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

To significantly demonstrate a difference in ANA between denervated and non-denervated animals we would need 8 animals (Power of Analysis 0.8). Assuming we will only manage to denervate 80% of nerve traffic, we will need a total of 10 animals in the non-denervated group (Aim 2) to adequately compare with denervated group. In aim 3, we will need 24 animals total, 8 in each group (afferent, efferent sympathetic and efferent sympathovagal) to reject then null hypothesis.

Total animals requested: 34

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.)

All canines will have an initial survival surgery where a pacemaker and radiotelemetry device will be implanted via left thoracotomy and trans venous access. 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.

Surgery will be performed under full aseptic technique. We will perform a left lateral thoracotomy incision at the T3-4 intercostal space to implant two devices. 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 to an experimental device [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 two iterations; the first iteration 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. The second iteration has two bipolar channels implanted into the stellate ganglion and vagus, and the third channel is a blood pressure recording channel which will be implanted into the subclavian artery. All leads will be sutured to the surrounding fascia and muscle layers in multiple locations to stabilize the leads in position.
The pacemaker and [redacted] 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. Right before closure, we will perform an EP study in order to determine the refractory period of the ventricle and inducibility of ventricular arrhythmias in a baseline state. For this portion, we would like to record nerve activity whilst performing the EP study as we would like to determine the mechanisms by which autonomic nerve activity is perturbed by PVCs and by ventricular arrhythmia. To record nerve activity, we will switch from isoflurane to pentobarbital anesthesia as isoflurane is suppressive of nerve activity whereas pentobarbital is less so. First,

pentobarbital 4-5mg/kg will be administered. Approximately five minutes later, isoflurane will be removed. Anesthesia will then be monitored to adequately maintain surgical plane of anesthesia by continuously monitoring of blood pressure and heart rate. If heart rate rises more than 5bpm, an additional small dose (no more than 2mg/kg) of Pentobarbital will be given. Total Pentobarbital per surgery will not exceed 30 mg/kg. Once a stable plane of anesthesia is achieved, an EP study is performed. This consists of giving isolated PVCs first. Second, we will apply single premature stimulus to determine the effective refractory period of the ventricle. Finally, we will apply double followed by triple premature stimulus to attempt to induce ventricular arrhythmia. In our experience in the baseline state, we do not expect any arrhythmia at all or at least only non-sustained ventricular arrhythmia (usually <10 beats). In case there is sustained ventricular arrhythmia that requires resuscitation, we will have defibrillation pads ready to terminate arrhythmia when necessary. At the end of this EP study (typically about 20 minutes), we will resume isoflurane and remove pentobarbital.

We will then proceed with closure of the chest wound. 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.

Most animals will follow the following recovery protocol at this point:

Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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 weekly until the animal completes the protocol.

There is a very small risk of spontaneous ventricular fibrillation during the left thoracotomy surgery. Should this happen there are sterile internal defibrillator paddlers connected to a defibrillator set at 50 Joules prepared for resuscitation efforts. Epinephrine will also be administered at a low dose (0.01 mg/kg) will be given every 3–5 min early in resuscitation efforts; a high dose (0.1 mg/kg) will be given after prolonged effort (15 minutes) with no response. Amiodarone (7mg/kg) will be used as an alternative. This is administered in a single dose and repeated as needed every 5 minutes.

The animals in the Thoracic Efferent denervation group will continue with this novel procedure before recovering from anesthesia:

This procedure will occur during the pacemaker implantation surgery (first surgery) described above. The animals in the Thoracic efferent denervation group will undergo a surgery (pre-operative procedures, analgesia described above) where we will cryoablate the caudal half of the left stellate ganglion (LSG) only, or the LSG and cardiac branch of the vagal nerve. The procedure is expected to add no more than 30 minutes of time to first surgery, with other parts of first surgery remaining the same as in all animals. Following cryoablation of nerves, the electrodes of the DSI device will be implanted cranial to the ablated portion to record nerve activity.

Under general anesthesia, a left thoracotomy as described above will be performed. Blood will be sampled simultaneously from the coronary sinus (CS) and aorta (AO) before and immediately after left stellate ganglion (LSG) stimulation (20s, 10mA, 20Hz, 2ms pulse width). Cryoablation (to minus 40°C for two minutes x 2 attempts) will then be performed of the caudal half of the LSG, and T3-4 sympathetic ganglia, and cardiac branch of the vagal nerve via cryo catheter. To confirm adequacy of ablation, we will electrically stimulate the nerves (20Hz, 2ms pulse width, 20s, 15mA) before and 5 minutes after each ablation. Cryoablation is considered complete when stimulation of each ablated nerve is no longer producing any changes of heart rate or blood pressure. DSI recording wires will be implanted to the unablated (upper) half of the LSG, the superior cardiac branch of the left vagal nerve cranial to the ablated portion and to left atrial (LA) epicardium. The wires are connected to a DSI transmitter, which is implanted into a subcutaneous pocket. The cryoablation procedure has previously been reported (Tan AY, Zhou S, Ogawa M, Song J, Chu M, Li H, Fishbein MC, Lin S-F, Chen LS, Chen P-S. Neural Mechanisms of Paroxysmal Atrial Fibrillation and Paroxysmal Atrial Tachycardia in Ambulatory Canines. Circulation 2008; 118(9):916-25. PMID: 18697820).

Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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 then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy after 2 weeks of recovery.

The side effect we have noted is transient horner's syndrome (pupillary dilation, drooping eyelid), as the fibers that supply pupillary motion passes through the cranial half of the stellate ganglion. By limiting ablation to the lower half, this side effect is transient.

Secondly, some animals have a limp for 1-3 days because of some nerves that supply the upper limb pass close by and sustain reversible cold injury. The veterinarian will monitor any limp and Carprofen can be administered if pain is noted. This limp appears to be due to numbness rather than pain.

However, all animals to date have recovered fully.

Sino-aortic Denervation Surgical Procedure (second and third survival surgery).

Sino-Aortic Afferent Nerves Denervation Part 1
This will be performed at least 3 weeks after first 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, 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

We will perform a midline dissection of the neck. Using this exposure, we will then dissect down to expose the carotid sinus nerve, and the aortic depressor nerve. The carotid sinus nerve is located between the external and internal carotid arteries. The aortic depressor nerve is contained within the vagus sheath, along with the vagus nerve. Ligation of both nerves, the carotid sinus nerve and aortic depressor nerve (not the vagus nerve) constitute sino-aortic denervation. We will identify the structures i.e., the vasculature (common carotid artery, internal and external carotid arteries), vagus nerve, and carotid body. We will perform an arteriotomy of the common carotid artery to insert a 7Fr sheath. Through this, we will insert a Millar catheter into the left ventricle for measurement of cardiac hemodynamics. We will then proceed towards denervation. We will ligate using silk sutures the fat and nerve tissues (not the arteries) between the internal and external carotid arteries at their origin from the common carotid artery. This will effectively denervate the carotid sinus afferent nerves. Secondly, we will use a dissecting microscope to locate aortic depressor nerve. This nerve is located within the vagus sheath that also contains the vagus nerve. We will ensure that the main vagus nerve is spared, and handling of the vagus nerve is minimized. We will ligate or ablate this using radiofrequency ablation 1 minute of radiofrequency ablation at 10W (maximum temperature 50 degree C). We will perform hemodynamic measurements before and after ablation, with and without PVCs. Finally, we will remove the arteriotomy by ligation of the common carotid artery. We will perform unilateral denervation first during second surgery. Muscle layers and subcutaneous tissues will be closed in multiple layers. Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/kg IM and monitored closely for adverse effects due to vagus nerve damage. This includes drooling, anorexia, vomiting and lethargy. The animals are visually monitored daily. Any side effects will be reported to the veterinarian.
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) can be given for 1-3 days if pain is noted 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). This procedure typically results in significant nausea

and IV fluids will be given to prevent dehydration. 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 then start chronic PVC exposure for the induction of PVC-induced cardiomyopathy.

Sino-aortic afferent nerves denervation part 2.
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

We will perform this at least 3 weeks after part 1 sino aortic denervation surgery. We will perform a midline dissection of the neck. We will reincise the original skin and subcutaneous incision used during part 1. Using this same exposure, we will then dissect down the opposing side from Part 1 from the second survival surgery (see above) to expose the carotid sinus nerve, and the aortic depressor nerve. The carotid sinus nerve is located between the external and internal carotid arteries. The aortic depressor nerve is contained within the vagus sheath, along with the vagus nerve. Ligation of the nerves, the carotid sinus nerve and aortic depressor nerve (not the vagus nerve) constitute sino-aortic denervation. We will identify the structures i.e., the vasculature (common carotid artery, internal and external carotid arteries), vagus nerve, and carotid body. We will then proceed towards denervation. We will ligate using silk sutures the fat and nerve tissues (not the arteries) between the internal and external carotid arteries at their origin from the common carotid artery. This will effectively denervate the carotid sinus afferent nerves. Secondly, we will use a dissecting microscope to locate aortic depressor nerve. This nerve is located within the vagus sheath that also contains the vagus nerve. We will ensure that the main vagus nerve is spared, and handling of the vagus nerve is minimized. We will ligate or ablate this using radiofrequency ablation 1 minute of radiofrequency ablation at 10W (maximum temperature 50 degree C). We will perform hemodynamic measurements before and after ablation, with and without PVCs. This will complete bilateral denervation during the third surgery. Muscle layers and subcutaneous tissues will be closed in 3 layers (interrupted PGA sutures for hypodermis, continuous PGA suture for the dermis, and a final interrupted nylon anchor suture layer to connect the epidermis. Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/kg IM and monitored closely for adverse effects due to vagus nerve damage. This includes drooling, anorexia, vomiting and lethargy. The animals are visually monitored daily. Any side effects will be reported to the veterinarian.

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) can be given for 1-3 days if pain is noted 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). This procedure typically results in significant nausea and IV fluids will be given to prevent dehydration. 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 monitored closely for adverse effects due to vagus nerve damage. They will then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy after 2 weeks of recovery.

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.

Pacemaker interrogation. After pacemaker implantation, device will be interrogated every 1-2 weeks 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 a St Jude Medical programmer. The programmer is and 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.

Autonomic Nerve recording. We will turn on the DSI to record baseline data for 1 week. We will then turn on pacemaker (and turn off the DSI) to rapidly pace the left ventricular (PVCs) to create electrical remodeling and promote CM. They will then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy (10 weeks) and then after, 2 weeks of recovery.

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.

Echocardiogram. 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 supine for at least 10 minutes

in order to obtain accurate cardiac images. Therefore, we believe that 2 different approaches will be required to obtain echocardiogram: 1) animal training with or without restraint, and/or 2) general anesthesia.
Our first approach will be animal training to stand and lay supine for 10 minutes to obtain echocardiogram. 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.

Baseline echocardiogram will be performed at least 2 weeks after pacemaker implantation and appropriate recovery of the animal. Subsequent echocardiograms will be performed on a monthly basis for the duration of the protocol. Echocardiogram will take from 10-20 minutes to obtain all required data. We estimate that each animal will undergo from 5 up to 12 echocardiograms depending on the assigned phase as per protocol.

Blood drawn – We will obtain blood sample in all groups at baseline as well on a monthly basis 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. Blood will be drawn from the brachial or jugular veins.

Intravenous pharmacological challenge. In order to understand and validate recordings in the autonomic nerves and relationship to heart rhythm and blood pressure, 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 imidazoline receptors in the midbrain, resulting in slow heart rate. It will initially cause a rise in blood pressure through action on peripheral alfa 2 receptors in the postsynaptic terminal resulting in vasoconstriction. Subsequently, it will lower blood pressure by acting on peripheral pre-synaptic alfa-2 receptors in sympathetic nerve terminals and by suppressing central sympathetic output. 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.
   It has been observed in the bilateral denervated animals (N=2), that after administering clonidine, there is more dramatic hypertension (than in non-denervated animals N=8 or in unilateral denervated animals, N=5), occurring within the first 15 minutes, followed by normalization of BP. We suspect this is due to denervated state. We observed short runs of nonsustained ventricular tachycardia (VT) (<3 sec) during this early period lasting about 15 minutes. We observed it in one animal, but not in another bilateral denervated animal. The observed animal did not experience any ill effects or appear distressed. After 15 minutes, hypertension and ventricular tachycardia subsided. BP recovered to normal (not less than 100mmHg systolic). To combat this,

the animal will be monitored for 60 minutes post administration. If sustained VT is observed on the DSI recording for more than 2 minutes, the animal will be administered Amiodarone (7mg/kg IV, every 5 minutes as needed). If hypotension occurs, we will administer fluids. Because this medication is transient, we do not expect sudden cardiac death to occur. We have not observed any evidence of dramatic hypotension as a result of this medication in either two animals.

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]. [Moise, N., Moon, P. F., Flahive, W. J., Brittain, D., Pride, H., Lewis, B. A., ... & Gilmour, R. F. (1996). Phenylephrine-Induced Ventricular Arrhythmias in Dogs with Inherited Sudden Death. *Journal of cardiovascular electrophysiology*, *7*(3), 217-230.]

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. We continuously monitor BP and heart rate via DSI recordings for 1 hour. The medications peak rapidly (within a few minutes), and its effects wane after about 20 minutes. By 1 hour, the vital signs are back to baseline.

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)
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).

It is possible for non-sustained ventricular arrhythmias to be induced with VPS, especially in a sick heart. 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, the animal will receive Carprofen 2mg/kg daily for one to three 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) after prolonged effort (around 15 minutes) with no response. Amiodarone (7mg/kg) will be used as an alternative. This is administrated in a single dose and repeated as needed every 5 minutes.

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 post-surgery, 2 weeks post-surgery before PVCs are started, at the end of the 12 week PVC period, and after 4 weeks of PVC recovery.

Treadmill. Canines will be exercised on a DogPACER (canine specific treadmill) to observe how PVCsPVCs are affecting the animals' exercise capacity, autonomic nervous recordings and possible arrhythmias. 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.
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.
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.
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 jugular or brachial vein. Blood will be drawn 4 times: once before, during each of 3 treadmill stages and recovery phase 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 jugular vein or brachial and skin taped comfortably around the dogs' neck or front leg to stay in place. If necessary because of a dog's personality, Acepromazine 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.

PVC-induced Cardiomyopathy model. Chronic PVC 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 Bigem 200 PVCs. This typically allows for the gradual development of cardiomyopathy without symptomatic congestive heart failure. Diazepam (0.02-2.0 mg/kg PO or IM) in canines will be administered as needed for distress. To date we have not had to administer this therapy, as no animal has exhibited signs of congestive heart failure.

Terminal surgery.

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 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 left thoracotomy or midline sternotomy 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. As stated previously for first surgery, the same EP study protocol is repeated in terminal surgery to determine the refractory period of the ventricle and inducibility of ventricular arrhythmias in a state of cardiomyopathy. Comparison is made with results from a baseline state, to determine the presence of electrical remodeling in the ventricle following the development of PVC-induced cardiomyopathy. For this portion, we would like to record nerve activity whilst performing the EP study as we would like to determine the mechanisms by which autonomic nerve activity is perturbed by PVCs and by ventricular arrhythmia.

In order to most accurately view autonomic nerve activity during an EP study, isoflurane may need to be turned off for a period of about 30-45 minutes. We will attempt to conduct these studies with isoflurane anesthesia but if it interferes with the readings, we will switch to pentobarbital anesthesia for a brief time. 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 using a butterfly needle. The dog will be exsanguinated under anesthesia by harvesting the heart. Tissues will be collected and preserved in 4% formadelhyde 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.

Model	Groups	No of animals	First surgery	Nerve recording	Chronic Cardiac Pacing	Second Denervation Surgery	Third Denervation surgery	Third Final Surgery
PVC-induced CM model	Controls	10	√	√	√			√
	Sino-aortic afferent denervation	8	√	√	√	√	√	√
	Thoracic efferent denervation (LCG only)	8	√	√	√	Completed during first surgery		√
	Thoracic Efferent Denervation (LSG and cardiac vagal)	8	√	√	√	Completed during first surgery		√
Totals		34						

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 (approximately 2 inches long, ¼ inch thick and 1.5 inch wide) electronic defibrillator / pacemaker, which has been specifically developed for our study. The radiotelemetry 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, that 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]

[redacted]

Qualifications to perform specific procedures

First Surgery	**Has 8 years experience with implanting pacemakers. Has completed all training modules with respect to canines, surgery and anesthesia**
Pacemaker interrogation	[redacted]
Echocardiogram	
Blood drawn	
Electrocardiogram	
Final/ Terminal Surgery	

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
	[redacted]
First Surgery	
Terminal/Final Surgery	

Name [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]
Afferent Denervation	
Efferent Nervation	
Pacemaker interrogation	
Autonomic Nerve recording.	
Echocardiogram	
Blood drawn	
Electrocardiogram	
Intravenous pharmacological challenge	
Ventricular programmed stimulation (VPS)	
Treadmill Exercise Challenge	
Cardiomyopathy model. Chronic Pacing protocol	
Terminal/Final Surgery	

Name▶ [redacted] **in animal care including surgical and non-invasisve procedures described in this project. He is currently enrolled in the Pre Med program at** [redacted] **University. No animal experience.**

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]
Afferent Denervation	
Efferent Nervation	
Pacemaker interrogation	
Autonomic Nerve recording.	
Echocardiogram	
Blood drawn	
Electrocardiogram	
Intravenous pharmacological challenge	
Ventricular programmed stimulation (VPS)	
Treadmill Exercise Challenge	
Cardiomyopathy model. Chronic Pacing protocol	
Final/Terminal Surgery	

Name► [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]
Afferent Denervation	

Efferent Nervation	
Pacemaker interrogation	
Autonomic Nerve recording.	
Echocardiogram	
Blood drawn	
Electrocardiogram	
Intravenous pharmacological challenge	
Ventricular programmed stimulation (VPS)	
Treadmill Exercise Challenge	
Cardiomyopathy model. Chronic Pacing protocol	
Final/Terminal Surgery	

Name►[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]
Afferent Denervation	
Efferent Nervation	
Pacemaker interrogation	
Autonomic Nerve recording.	
Echocardiogram	
Blood drawn	
Electrocardiogram	
Intravenous pharmacological challenge	
Ventricular programmed stimulation (VPS)	
Treadmill Exercise Challenge	
Cardiomyopathy model. Chronic Pacing protocol	
Final/ Terminal Surgery	

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]
Afferent Denervation		
Final/ Terminal Surgery		

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]		

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] will assist during the sino-aortic denervation. He has experience with this procedure in acute studies.

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]	

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 ►

► (√) No.

Animals Requested

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

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Canines, mongrel	**Female**	**6-12 months/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 / Procedure(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 and Bilateral Denervation						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**
PVC induced CM Model	**11**	**11**	**12**			**34**

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
	11	**11**	**12**			**34**

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.

► (√) 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 appendix 5**	[redacted]	**See appendix 5**
Efferent Denervation (LSG ONLY)	**see appendix 5**	[redacted]	**see appendix 5**
Efferent Denervation (LSG and vagal)	**see appendix 5**	[redacted]	**see appendix 5**
Terminal surgery	**see appendix 5**	[redacted]	**see appendix 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.
►

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 <3 sec in our experience. 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.

Bilateral Afferent Denervation (part 1 and 2)
After the denervation procedures, animals will experience the following side effects that may cause distress; nausea, vomiting and anorexia caused by transient injury to the vagal nerve. We provide anti-emetics, IV fluids and special foods. However, these side effects can only be ameliorated by anti-emetics but cannot be completely alleviated. We anticipate they will completely recover within 3-7 days. If they do not recover or their weight is reduced by more than 12%, they will be euthanized by pentobarbital overdose or exsanguination under anesthesia during a final procedure described above.

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:

a. Species	b. Type of housing*	c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
Canines	**Chain link run, 3x6 and 4x10 feet cage**	**1**	**no**	**7**

*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
Canines	10 minute exercise regimen daily, standard, see below	Standard, see below

*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.
►

► (√) 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).

► ( ) 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]	(√)	( )
		( )	( )
		( )	( )

► ( ) 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".

► (√) 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?

► (√) 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)**
Radiofrequency ablation of renal nerves	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Bilateral Afferent Denervation (part 1 and 2)	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Efferent Denervation (LSG ONLY)	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Efferent Denervation (LSG and vagal)	**(X)**	**( )**	[redacted]		
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	**( )**	**( )**	**(√)**	**( )**
Heart	**(√)**	**( )**	**( )**	**( )**
Pacemaker and Data Sciences International (DSI) device.	**(√)**	**( )**	**( )**	**( )**

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.)

1. If animals suffer from heart failure, they will satisfy end point and euthanasia will be performed. Signs include weight loss of more than 12%, not eating, lethargy, weight gain due to fluid retention, signs of fluid accumulation in prone locations, in the case of canines, in the abdomen (ascites). 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. Blood pressure will also be monitored weekly and recorded in the chart.
2. Alternatively, if they were to suffer a serious postoperative complication such as uncontrollable bleeding due to tears to blood vessels such as carotid artery, aorta, laceration of the cardiac chambers or pulmonary vasculature, injuries to lung parenchyma such as pneumothorax, hemothorax, renal infarction (presenting as hematuria, pain, renal failure manifested by oliguria, anuria, fatigue, listlessness) as a result of occlusion of renal artery, end point criteria will be reached.
3. Other intraoperative end points include: ventricular fibrillation which is refractory to resuscitation. This can occur with lead fixation into ventricle, or during EP study.

4. If there is uncontrollable infection of the pocket in which pacemaker and DSI are placed, and these cannot be controlled by antibiotics, then end point criteria is reached. Pocket infection presents as pocket swelling with purulence, erythema, pain, fever, anorexia, which does not resolve with 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.
5. Finally, if there is unrecoverable peripheral nerve damage from cryoablation, which causes the animal to have permanent limb paresis, which results in not being able to weight bear, self-mutilation of the limb that is not treatable by any means.

During the second and third survival surgery, there is a risk of over stimulating the nerve such that the animal may have unrecoverable severe nausea and gastroparesis. The PI and animal technician will be responsible for monitoring the animal closely after this surgery. If needed, fluids will be administered twice daily, a variety of foods (dry food, wet food, baby food, and nutritional supplement) will be provided to stimulate appetite, and anti-emetics (Bismuth subsalicylate, famotidine, metoclopramide) will be delivered IV as prescribed by the veterinarian to counter the nausea. If the animal shows no signs of improvement after 72 hours of treatment, the animal will be euthanized by pentobarbital overdose.

All animals will have daily weights recorded while on antibiotics or undergoing any other treatment prescribed by the veterinarian. They will be weighed twice weekly otherwise.

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►100mg/kg Route of administration►IV	canine	(X)	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
(√)	Exsanguination under anesthesia Agent► Isoflurane Dose► 1-3% Route of administration► inhalation	Canine	(√)	( )	( )
( )	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.
   ►[redacted] [redacted] **and** [redacted] **will perform euthanasia. All have extensive experience in canine surgery of the nature described for second surgery.**

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] **is to be informed immediately. 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.
► (X) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
Name► [redacted]

Contact Information►
► ( ) 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)**
Treadmill Exercise		Items: C.2.c	( )**
Nerve Recording		Items:	(x)**
Electrocardiogram		Items: C.2.c	
Blood Draw		Items: C.2.c	
VPS		Items: C.2.c	
Echocardiogram		Items: C.2.c	

*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.
► thoracotomy, denervation, vagal injury, VPS, atrial fibrillation

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

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
NIH PubMed	05/24/2017	2016-2017	first surgery; device pocket swelling, ablation of survival thoracotomy, autonomic denervation, vagal injury, exercise challenge, aortic depressor nerve	ablation, sympathetic, atrial fibrillation, arrhythmia, autonomic, vagal nerve injury,	(X)	(X)	(X)	(X)
ALTWEB	05/24/2017	2016-2017	first surgery; device pocket swelling; ablation of terminal surgery, infarction, survival thoracotomy, autonomic denervation,	ablation, sympathetic, atrial fibrillation, arrhythmia, autonomic, vagal nerve injury	(X)	(X)	(X)	(X)

			vagal injury, aortic depressor nerve					
					( )	( )	( )	( )
					( )	( )	( )	( )

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.
► Canines have very similar physiology to humans. The recently developed model for experimental CM is the most suitable model for this project. Smaller animals like rodents and rabbits are not suitable models for CM as their atrial chambers are too small to sustain AF, and there are significant differences in cardiac physiology between small animal species and humans. Other large animals such as pigs are not as suitable for chronic instrumentation from a behavioral standpoint. It would be difficult to record cardiac and autonomic nerves long term simultaneously in a small animal as their nerves are too small to be recorded with the materials and technique that is being utilized by the P.I. The P.I. also has extensive experience with long-term autonomic nerve recording in canines. 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 [redacted] **to undergo pacemaker interrogation with minimal distress.**
The study of changes in LV function and cardiac contractility due to frequent PVCs and PACs is unknown and therefore, there are no computer models to answer the unknown questions. We have 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. 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.
► Multiple interventions will be performed in the same animal in sequential fashion to reduce the number of animals used. Each animal will act as his own control (baseline recording before atrial pacing or radiofrequency catheter ablation of renal nerves). Therefore, the need for a separate control group is eliminated.

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

[redacted]

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
► Simultaneous long term recording of renal and cardiac autonomic nerves has never been attempted and is the novel aspect of this study. Additionally, the effects of ablation of renal sympathetic nerves on cardiac physiology has never been studied in detail in an animal model.

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)	( )*		(X)	( )	(X)	( )
Brevital	(X)	( )*		(X)	( )	(X)	( )
Diazepam	(X)	( )*		(X)	( )	(X)	( )

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

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

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

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

► ( ) Other. Explain►

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

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

► (√) 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".

► (√) 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. <u>Do not check off or attach any appendices that are not applicable to this ACORP.</u>

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (X) Appendix 3, "Biosafety"
► (X) 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	SOP		
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
[redacted]		
Name of IACUC Chair	Signature	Date
[redacted]		

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
[redacted]		
Name of IACUC Chair	Signature	Date
[redacted]		

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]

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
[redacted]		
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of VISN Regional Safety Officer	Signature	Date

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

ACORP APPENDIX 3
BIOSAFETY
VERSION 4

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

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

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

Carprofen	Butler Schein	( )					(X)	
Cefpodoxime	Butler Schein	( )					(x)	
Baytril	Bayer	( )					(X)	
Penicillin	Butler Schein	( )					(X)	
Bismuth Subsalicylate	Local pharmacy						(X)	
Famotidine	Local pharmacy						(X)	
Metoclopramide	Richmond VA Pharmacy						(x)	
Meloxicam	Butler Schein						(X)	
Epinephrine	Richmond VA pharmacy							
Amiodarone	Richmond VA Pharmacy							
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 per 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
Durapen	3ml		SC	Once	Antibiotics	App5	N
Cefpodoxime	5mg/kg		PO	Daily	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	N

Metoclopromide	0.2-0.5 mg/kg		IV	PRN	nausea	App 5	N
Phenylephrine	0.01mg/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	N
Brevital	6-10 mg/kg to effect	Normal saline	IV	Once for 1 day	Sedation during surgery	App5	N
Diazepam	0.2-2.0 mg/kg		Oral or IM	up to Twice a day PRN	Calm during post-op to keep sutures intact	App5	N
Carprofen	2 mg/kg		Oral	SID up to 3 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	Y
Bismuth Subsalicylate	262 mg	None	Oral	Once a day as needed	Appetite recovery	App5	N
Famotidine	0.22-0.44 mg/pound	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	7mg/kg	None	IV bolus	Single dose, repeated every 5 minutes if necessary	Resuscitation	App5	Y and N	
Vetericyn gel spray	2-3 sprays	None	Topical	Once per surgery	To 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 are pharmaceutical grade

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 and Pacemaker are implanted 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

*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)	( )	( )	( )**

*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**

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 member is at risk**
Phenylephrine	**SRS**	**VA**	**No Staff member is 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.
►There are no risks to staff members.

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**	**10-15 ml (< 1%)**	**No**	**5**	**Monthly**
Blood	**Aorta or coronary sinus**	**Yes**	**>10ml**	**Yes**	**Twice**	**Several months**

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).

► **No tranquilizers will be required since the pain will be momentary. Administration of tranquilizers or anesthetics may cause similar discomfort or pain caused by phlebotomy.**

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

► Approved personnel will briefly restrain the animal during IV injection by holding off the jugular vein with one hand and holding the animal's head with the other. This restraint will last less than 30 seconds and will not distress the animal.

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	0.05-0.1mg/kg	**Oral**	**Once**

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.
►

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).
►

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).
►

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	Efferent Denervation (LSG only)	( )	( )	(X)	()*
3	Efferent Denervation (LSG and Vagal)	( )	()	(X)	()*
4	Bilateral Afferent Denervation Part 1	( )	()	(X)	(X)*
5	Bilateral Afferent Denervation part 2	(√)	( )	(X)	(X)*
6	Terminal/ Final Surgery	( )	( )	(X)	(X)*
7	Lead Revision			(X)	(X)
8	Wound Revision		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 <u>complete scientific justification</u> for performing the multiple survival surgeries on an individual animal:
► Performing a second survival surgery is meant for radiofrequency catheter ablation or ligation of afferent nerves (sinoaortic denervation) This is minimally invasive. The only incision involves a midline skin only cervical incision, followed by muscle plane separation (without muscle incision). This portion

could not be performed during first surgery as first surgery is a thoracotomy. If unable to collect data in the baseline state, we would not have internal controls which would necessitate a separate control group, and result in an increase in the number of animals used.

For the group of animals that are assigned to the bi-lateral afferent denervation, they will undergo two additional survival surgeries for the purpose of bilateral sino-aortic denervation. These surgeries involve minimal incision in the midline neck. The staged procedure is required so as to allow the animal to recover from any inadvertent (though reversible) vagus nerve injury during the process of surgical procedure to perform sino-aortic denervation. The vagus nerve is not the target, but it is a possible collateral injury. It is expected that any vagus nerve injury is reversible as the vagus nerve is not ligated. No animal will undergo bilateral sino aortic denervation simultaneously.. These are two separate denervation procedures in order to minimize adverse effects of performing simultaneous bilateral denervation. Due to the potential for serious physiological impairment, this has been classified as a major surgery.

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.

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):
►

There is an interval of approximately 21-28 days between first surgery and the first unilateral sino-aortic denervation. After this there is additional 21-28 days between the first part and second part of the afferent bilateral denervation. The period between first and second surgery is because of recovery from major thoracic incision. There is an interval of approximately 21-28 days between second surgery and the third surgery (contralateral sino-aortic denervation).The second and third surgeries are marked by the same midline skin incision only. There are no muscle incisions. The observation period of 21 to 28 days is intended to monitor for vagus nerve injury side effects (if any), not for healing from the surgical incision per se. Note that in our experience, the animals have recovered to normal by the 2 week mark after thoracotomy and cervical incision surgery, thus we are confident that 21-28 days is adequate recovery for them prior to the next surgery.
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.

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. <u>First survival surgery.</u>

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 to implant two devices. The heart will be subsequently exposed via transection and retraction of the pericardial sac. While the epicardial surface is exposed we will implant a [redacted] in the right ventricular (RV) apex. These leads will be connected to [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 collect blood from the coronary sinus (CS) and aorta (AO). We will use a small butterfly needle for direct sampling of about 3cc blood each, followed by manual hemostasis.

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 either a bipole for epicardial electrocardiograms or a blood pressure catheter to be implanted into the subclavian artery. One lead will be attached to the anterolateral ventricular surface and the second lead is attached to the left atrial appendage or it will go in to the subclavian artery. All leads will be sutured to the surrounding fascia and muscle layers in multiple locations to stabilize the leads in position. The blood pressure catheter will be implanted into the subclavian artery close to its origin in the aorta in the thoracic cavity. Silk zero sutures will be placed proximal and distal to the arteriotomy site. The sutures will form a sling that can be lifted up to achieve temporary hemostasis, as the blood pressure catheter is placed into the artery via the arteriotomy. Upon insertion of the catheter into the artery, the proximal and distal sutures are tightened and tied to achieve hemostasis.

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.

Right before closure, we will perform an EP study in order to determine the refractory period of the ventricle and inducibility of ventricular arrhythmias in a baseline state. For this portion, we would like to record nerve activity whilst performing the EP study as we would like to determine the mechanisms by which autonomic nerve activity is perturbed by PVCs and by ventricular arrhythmia. To record nerve activity, we may have to switch from isoflurane to pentobarbital anesthesia as isoflurane is suppressive of nerve activity whereas pentobarbital is less so. First, pentobarbital 4-5mg/kg will be administered. Approximately five minutes later, isoflurane will be removed. Anesthesia will then be monitored to adequately maintain surgical plane of anesthesia by continuously monitoring of blood pressure and heart rate. If heart rate rises more than 5bpm, an additional small dose (no more than 2mg/kg) of Pentobarbital will be given. Total Pentobarbital per surgery will not exceed 30 mg/kg. Once a stable plane of anesthesia is achieved, an EP study is performed. This consists of giving isolated PVCs first. Second, we will apply single premature stimulus to determine the effective refractory period of the ventricle. Finally, we will apply double followed by triple premature stimulus to attempt to induce ventricular arrhythmia. In our experience in the baseline state, we do not expect any arrhythmia at all or at least only non-sustained ventricular arrhythmia (usually <10 beats). In case there is sustained ventricular arrhythmia that requires resuscitation, we will have defibrillation pads ready to terminate arrhythmia when necessary. At the end of this EP study (typically about 20 minutes), we will resume isoflurane and remove pentobarbital.

We will then proceed with chest wound closure. 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.

Most animals will follow the recovery protocol at this point:

Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incision will be sprayed with a Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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 weekly until the animal completes the protocol.

There is a very small risk of spontaneous ventricular fibrillation during the left thoracotomy surgery. Should this happen there are sterile internal defibrillator paddlers connected to a defibrillator set at 50 Joules prepared for resuscitation efforts. Epinephrine will also be administered at a low dose (0.01 mg/kg) will be given every 3–5 min early in resuscitation efforts; a high dose (0.1 mg/kg) will be given after prolonged effort (15 minutes) with no response. Amiodarone (7mg/kg) will be used as an alternative. This is administered in a single dose and repeated as needed every 5 minutes.

The animals in the Thoracic Efferent denervation group will continue with one of the following novel surgical procedures:

Efferent Cardiac Denervation (LSG only)

This will be performed in one group of animals during as described above and will occur after the implantation of the two devices.. Under general anesthesia, a left thoracotomy as described above will be performed in 8 dogs. Blood will be sampled simultaneously from the coronary sinus (CS) and aorta (AO) before and immediately after left stellate ganglion (LSG) stimulation (20s, 10mA, 20Hz, 2ms pulse width). Cryoablation (to minus 40^{o}C) will then be performed of the caudal half of the LSG, via cryo catheter. To confirm adequacy of ablation, we will electrically stimulate the nerves (20Hz, 2ms pulse width, 20s, 15mA) before and 5 minutes after each ablation. Cryoablation is considered complete when stimulation of each ablated nerve is no longer producing any changes of heart rate or blood pressure. DSI recording wires will be implanted to the unablated (upper) half of the LSG, the superior cardiac branch of the left vagal nerve cranial to the ablated portion and to left atrial (LA) epicardium. The wires are connected to a DSI transmitter, which is implanted into a subcutaneous pocket. A pacing lead will be implanted onto the LA appendage (LAA) and connected to a subcutaneously positioned [redacted] atrial pacing (20Hz, twice diastolic threshold, 2ms pulse width). Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with a Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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 (alternatively if this isn't effective, 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.
The animal will then be recovered for 2 weeks before they will then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy. Note that everything else described for first surgery will remain unchanged.

Efferent Cardiac Denervation (LSG and Thoracic Cardiac Vagal)

This will be performed in one group of animals during as described above and will occur after the implantation of the two devices. Under general anesthesia as described above, a left thoracotomy will be performed in 8 dogs. Blood will be sampled simultaneously from the coronary sinus (CS) and aorta (AO) before and immediately after left stellate ganglion (LSG) stimulation (20s, 10mA, 20Hz, 2ms pulse width). Cryoablation (to minus 40^{o}C) will then be performed of the caudal half of the LSG, and T3-4 sympathetic ganglia, and cardiac branch of the vagal nerve via cryo catheter. To confirm adequacy of ablation, we will electrically stimulate the nerves (20Hz, 2ms pulse width, 20s, 15mA) before and 5 minutes after each ablation. Cryoablation is considered complete when stimulation of each ablated nerve is no longer producing any changes of heart rate or blood pressure. DSI recording wires will be implanted to the unablated (upper) half of the LSG, the superior cardiac branch of the left vagal nerve cranial to the ablated portion and to left atrial (LA) epicardium. The wires are connected to a DSI transmitter, which is implanted into a subcutaneous pocket. A pacing lead will be implanted onto the LA appendage (LAA) and connected to a subcutaneously positioned [redacted] pacing (20Hz, twice diastolic threshold, 2ms pulse width). The animal will then be recovered for 2 weeks before they will then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy. Note that everything else described for first surgery will remain unchanged. Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with a Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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 (alternatively if this isn't effective, 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.

Note that only the cardiac branch of the vagus efferent will be ligated, which means that we do not expect GI side effects. The PI's experience with this model validates the technique. There were no noticeable GI side effects in 8 animals previously studied by the PI. The efferent denervation can result in a limp that seems to be attributed to numbness and not pain. The animal will be monitored and the veterinarian consulted. Carprofen can be provided if necessary. In our experience this resolves within 5 days.

The animals undergoing the Efferent cardiac denervation procedures do not continue to the afferent denervation procedures. They will only have one survival surgery.

For the animals that completed the first thoracotomy without the efferent denervation, they will be allowed to recover for 21-28 days and then complete the following two afferent denervation surgeries.

3. **Afferent Denervation Part 1.**

This surgery will be performed in a subset of animals.. The animals continuing with the afferent denervation will have completed the initial pacemaker and DSI device implantation surgery 21-28 days prior. 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

We will perform a midline dissection of the neck. Using this exposure, we will then expose the right carotid sheath. We will identify the structures within this sheath, i.e., the vasculature (common carotid artery, internal and external carotid arteries), vagus nerve, and carotid body. We will perform an arteriotomy of the common carotid artery to insert a 7Fr sheath. Through this, we will insert a Millar catheter into the left ventricle for measurement of cardiac hemodynamics. We will then proceed towards denervation. Prior to denervation, Pentobarbital 4-5mg/kg will be administered. Approximately five minutes later, isoflurane will be removed. Anesthesia will then be monitored to adequately maintain surgical plane of anesthesia by continuously monitoring of blood pressure and heart rate. If heart rate rises more than 5bpm, an additional small dose (no more than 2mg/kg)of Pentobarbital will be given. Total Pentobarbital per surgery will not exceed 30 mg/kg. Once a stable plane of anesthesia is achieved, we will ligate using silk sutures the fat and nerve tissues (not the arteries) between the internal and external carotid arteries at their origin from the common carotid artery. This will effectively denervate the carotid sinus afferent nerves. Secondly, we will use a dissecting microscope to locate the aortic depressor nerve which can sometimes branch off the vagus trunk, sometimes off the cervical sympathetic nerve, sometimes be a separate nerve in itself. We will ablate ligate this. We will minimize any mechanical handling of the vagus trunk in order not to injure it. We will perform hemodynamic measurements before and after ablation, with and without PVCs. We will then resume isoflurane and discontinue Brevital. Finally, we will close the arteriotomy by ligation of the common carotid artery. We will perform unilateral denervation (and only unilateral arteriotomy for insertion of hemodynamic catheter into the left ventricle. Muscle layers and subcutaneous tissues will be closed in 3 layers (interrupted PGA sutures for the muscle layer, continuous PGA suture for the subcutaneous layer, and interrupted Nylon suture for the final anchor layer).

The animal will then be recovered. Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with a Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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.

The animal will be allowed to recover 21-28 days before proceeding with part 2 to complete bilateral denervation.

4. **Afferent Denervation part 2**

This surgery will be performed 21-28 days post part one of the afferent denervation 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, 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.

We will perform a midline dissection of the opposing side of the neck done in the previously described part 1 of this surgery. Using this exposure, we will then expose the left carotid sheath. We will identify the structures within this sheath, i.e., the vasculature (common carotid artery, internal and external carotid arteries), vagus nerve, and carotid body. We will perform an arteriotomy of the common carotid artery to insert a 7Fr sheath. Through this, we will insert a Millar catheter into the left ventricle for measurement of cardiac hemodynamics. We will then proceed towards denervation. Prior to denervation, 5mgkg Pentobarbital will be administered. Approximately five minutes later, isoflurane will be removed. Anesthesia will then be monitored to adequately maintain surgical plane of anesthesia by continuously monitoring of blood pressure and heart rate. If heart rate rises more than 5bpm, an additional small dose of Pentobarbital (no more than 2mg/kg) will be given. Total pentobarbital per surgery will not exceed 30mg/kg. Once a stable plane of anesthesia is achieved, we will ligate using silk sutures the fat and nerve tissues (not the arteries) between the internal and external carotid arteries at their origin from the common carotid artery. This will effectively denervate the carotid sinus afferent nerves. Secondly, we will use a dissecting microscope to locate the aortic depressor nerve which can sometimes branch off the vagus trunk, sometimes off the cervical sympathetic nerve, sometimes be a separate nerve in itself. We will ablate ligate this. We will minimize any mechanical handling of the vagus trunk in order not to injure it. We will perform hemodynamic measurements before and after ablation, with and without PVCs. We will then resume isoflurane and discontinue Brevital. This will complete the bilateral denervation arteriotomy for insertion of hemodynamic catheter into the left ventricle. Muscle layers and subcutaneous tissues will be closed in 3 layers (interrupted PGA sutures for the muscle layer, continuous PGA suture for the subcutaneous layer, and interrupted Nylon suture for the final anchor layer).

Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/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) can be given for 1-3 days if pain is noted 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.
The animal will then be recovered for 2 weeks before they will then undergo chronic PVC exposure for the induction of PVC-induced cardiomyopathy.

6. 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, 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

A left thoracotomy or midline sternotomy 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 may be turned off for a period of about 30-45 minutes. We will attempt to do the EP study under isoflurane anesthesia but if there is interference we will give 5mg/kg of pentobarbital at this time prior to suspension of isoflurane and 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 that is allowed to prevent them from overdose. Once a stable plane of anesthesia is achieved, an EP study is performed. This consists of giving isolated PVCs first. Second, we will apply single premature stimulus to determine the effective refractory period of the ventricle. Finally, we will apply double followed by triple premature stimulus to attempt to induce ventricular arrhythmia. In our experience in the baseline state, we do not expect any arrhythmia at all or at least only non-sustained ventricular arrhythmia (usually <10 beats). In case there is sustained ventricular arrhythmia that requires resuscitation, we will have defibrillation pads ready to terminate arrhythmia when necessary. At the end of this EP study (typically about 20 minutes), we will resume isoflurane and remove pentobarbital.
Once the EP study and 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 harvesting the heart. Tissues will be collected and preserved in 4% formadelhyde 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.

7. 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 involve a thoracotomy surgery. Procedures, surgical technique and recovery would be the same as section "a".

8. **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 and Carprofen 2mg/kg for analgesia. 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. Vetericyn gel will be sprayed on the incisions. 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 #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	**1,2,3, 4, 5,6,7,8**	**(√)**	**(√)**	**( )**	( )
[redacted]	**1,2,3, 4, 5,6,7,8**	**(√)**	**(√)**	**( )**	( )
[redacted]	**1,2,3, 4, 5,6,7,8**	**( )**	**(√)**	**(√)**	( )
[redacted]	**1,2,3, 4, 5,6,7,8**		**(□)**	**(□)**	
[redacted]	**1,2,3, 4, 5,6,7,8**	**( )**	**(√)**	**(√)**	( )

[redacted]	**1,2,3, 4, 5,6,7,8**	**( )**	**(√)**	**(√)**	( )

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 not Dedicated to Surgery
[redacted]	[redacted]	**1,2,3, 4,5,8**	**(√)**	( )*	( )*
			()	(*)	( )*

*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	(√) – 12 hours	( )	(√) – brachial v	( ) --
2	(√) -- 12 hours	( )	(√) -- brachial v	( ) --
3	(√) – 12 hours	( ) –	(√) – brachial v	( ) --
4	(√) – 12 hours	( ) --	(√) – brachial v	( ) --
5	(√) – 12 hours		(√) – brachial v	
6	(√) – 12 hours		(√) – brachial v	
7	(√) – 12 hours		(√) – brachial v	
8	(√) – 12 hours		(√) – 12 hours	

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	1,2, 3, 5,6,7	0.01-0.02 mg/kg	IM	one	Immediate
Acepromazine	1,2,3,4,5,6,7,8	0.05-0.1mg/kg	PO	Once	1 hour before
Pentobarbital–survival surgery	1,2, 3,4,5,6,7	Up to 30mg/kg to effect	IV	Once if Brevital not available	immediate
Brevital	1,2,3,5,6,7,8	6-10 mg/kg to effect	IV	Once	immediate
penicillin	1,6,7	900,000 units	IM	Once	immediate
famotidine	1,2.3.5,6,7,8	0.5-1.0 mg/kg	oral	once	immediate
Carprofen	8	2mg/kg	Oral	Once	1 hour before

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 from the ventral part of the neck from the top of the chin, down the chest and prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.**

Surgery 3 ►**.**
Hair to be clipped from the ventral part of the neck from the top of the chin, down the chest and prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.

Surgery 4
Hair to be clipped from the ventral part of the neck from the top of the chin, down the chest and prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.

Surgery 5-
Hair to be clipped from the ventral part of the neck from the top of the chin, down the chest and prepped with betadine. All survival procedures will be done using sterile techniques and surgical drapes.

Surgery 6
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 7
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 8► **Fur will be shaved from all surgical sites. The sites will be scrubbed with betadine and 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	1,2.3.5,6,7,8	1-3% or 2-4% for mask	Inhalation	Continuous
Pentobarbital	No	1,2.3.5,6,7	Up to 30mg/kg to effect	IV	Once before surgery and once during surgeries when isoflurane is removed
Brevital	No	1,2.3.5,6,7,8	6-10 mg/kg to effect	IV	Once
Epinephrine	No	1,2,3,4,5,6,7	Low dose (0.01 mg/kg) every 3–5 min early; high dose (0.1 mg/kg) after prolonged; 1 ml	IV	Low dose: Every 3-5 mins High Dose: After prolonged VF (15 mins)
Amiodarone	No	1,2.3.5,6,7	7mg/kgmg bolus, repeated every 5 minutes as necessary	IV	Bolus administration, repeated every 5 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 during surgery (e.g., warming, cushioning, etc.).

The dog will be placed on a warming pad and blankets will be placed over extremities and lower half of the 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. 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). 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.

10. **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	20-24 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
2	20-24 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
3	20-24 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(√)*

4	20-24 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
5	20-24 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
5	terminal surgery	(X)	(X)	(X)	(X)	(X)	(X)	(X)	)*
7	12-16 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	)*
8	12-16 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, 5, 7, and 8
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)
1,2,3,4,5,7	Buprenorphine	0.01-0.02 mg/kg	IM	BID	1-3 days
1,2,3,4,5,7,8	Carprofen	2mg/kg	Oral	SID	7 days or as needed
1,2,3,4,5,7,8	Diazepam	0.2-0.4 mg/kg	Oral or IM	BID if needed	1-7 days
1,2,3,4,5,7	meloxicam	0.2mg/kg	IM or SQ	Once immediate post opeand as alternative to Carprofen	1 day

*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)
1,2,3,4,5, 7	bismuth subsalicylate	262mg	oral	SID	As needed
1,2,3,4,5, 7	famotidine	0.5-1.0mg/kg	oral	SID	As needed
1,2,3,4,5, 7	Metaclopramide	0.5 mg/kg	IV	SID	As needed
1,2,3,4,5,7,8	Cefpodoxime	5 mg/kg	Oral	Daily, as needed for wound infection	10 days
1,2,3,4,5,7,8	Baytril	5-20 mg/kg	Oral	Daily instead of cefpodoxime	10 days

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>.

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Continuously	1-2 hours post sternal	[redacted]
2	Continuously	1-2 hours post sternal	[redacted]
3	Continuously	1-2 hours post sternal	[redacted]
5	Continuously	1-2 hours post sternal	[redacted]
6	NA		
7	Continuously	1-2 hours post sternal	[redacted]
8	Continuously	1-2 hours post sternal	[redacted]

(2) 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	6-12 hours	3-4 days	[redacted]
2	6-12 hours	2-3 days	[redacted]
3	6-12 hours	3-4 days	[redacted]

5	6-12 hours	3-4 days	[redacted]
6	NA		
7	6-12 hours	3-4 days	[redacted]
8	2-6 hours	1-2 days	[redacted]

f. Post-operative consequences and complications.

(1) 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). The PIs have learned a technique from a Cardiothoracic surgeon to repair lung lacerations but this still may not be survivable. 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. Additionally, during the thoracotomy, rib fractures may occur. If this occurs, the veterinarian will be notified and Carprofen given for the 7 days after buprenorphine.
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. Mild hypertension may occur as a consequence of pain, therefore, this will be treated by analgesics (buprenorphine or meloxicam. Hypertension to a mild degree (<200/100mmHg) will not be otherwise symptomatic. Severe hypertension is not expected and has not been observed.

Surgery 2 and 3 (efferent cardiac denervation during first surgery)►.
During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). The PIs have learned a technique from a Cardiothoracic surgeon to repair lung lacerations but this still may not be survivable. 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.
Additionally, during the thoracotomy, rib fractures may occur. If this occurs, the veterinarian will be notified and Carprofen given for the 7 days after buprenorphine.

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. Hypertension may occur as a consequence of pain; therefore, this will be treated by analgesics (including buprenorphine or meloxicam). Hypertension to a mild degree (<200/100mmHg) will not be otherwise symptomatic. Severe hypertension is not expected and has not been observed.
Additionally, there is a risk of injuring the vagus nerve by excessive mechanical manipulation such that the animal may have unrecoverable severe nausea and gastroparesis post operatively.. The animal had transient horner's syndrome (droopy eyelids). The PI and animal technician will be responsible for monitoring the animal closely after this surgery. If the animal is anorexic or has reduced appetite, fluids will be administered twice daily, a variety of foods (dry food, wet food, baby food, and nutritional supplement) will be provided to stimulate appetite, and anti-emetics will be delivered IV as prescribed by the veterinarian to counter the nausea. If the animal shows no signs of improvement after 72 hours of treatment, the animal will be euthanized by pentobarbital overdose.

An additional post-operative risk is some collateral nerve damage that may result in paresthesia and weakness that may manifest as limping on the forelimb. This is anticipated to be reversible. The animal will be closely monitored until symptoms are alleviated and treated with 2mg/kg Carprofen orally. If symptoms persist for more than 7 days, the veterinarian will be consulted immediately for evaluation and further action.

Surgery 4 and 5 (afferent denervation parts 1 and 2) ► During this surgery, there is a risk of over stimulating the nerve such that the animal may have severe nausea and gastroparesis. The PI and animal technician will be responsible for monitoring the animal closely after this surgery. If the animal is anorexic or has reduced food intake, fluids will be administered twice daily, a variety of foods (dry food, wet food, baby food, and nutritional supplement) will be provided to stimulate appetite, and anti-emetics will be delivered IV and PO as prescribed by the veterinarian to counter the nausea. The animal's weight will be closely monitored. If the animal shows no signs of improvement after 72 hours of treatment, the animal will be euthanized.
An additional post-operative risk is some collateral nerve damage that may result in paresthesia and weakness that may manifest as limping on the forelimb. This is anticipated to be reversible. The animal will be closely monitored until symptoms are alleviated and treated with 2mg/kg Carprofen orally. If symptoms persist for more than 7 days, the veterinarian will be consulted immediately for evaluation and further action.
Finally, we wish to add potential complications related to unexpected deleterious effects of autonomic denervation on animals with PVC induced cardiomyopathy. We are testing the hypothesis that autonomic denervation may retard or prevent development of PVC-induced cardiomyopathy, however, there is a possibility that it may be harmful. It may be proarrhythmic in the setting of PVC-induce cardiomyopathy or accelerate development of cardiomyopathy with PVCs. Therefore, we may have unexpected deaths that we do not expect in this pilot protocol. This applies to the animal that have undergone surgeries 5,6 (afferent denervation) or to animals that have undergone surgeries 2 and 3 (efferent denervation).

Surgery 6 ► Terminal Surgery

Surgery 7-
During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). The PIs have learned a technique from a Cardiothoracic surgeon to repair lung lacerations but this still may not be survivable. 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.
Additionally, during the thoracotomy, rib fractures may occur. If this occurs, the veterinarian will be notified and Carprofen given for the 7 days after buprenorphine.

Major concern postoperatively 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.
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, including iv metoclopramide or po famotidine.
Hypertension may occur as a consequence of pain, therefore, this will be treated by analgesics (including buprenorphine or meloxicam). Hypertension to a mild degree (<200/100mmHg) will not be otherwise symptomatic. Severe hypertension is not expected and has not been observed.

Surgery 8-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).

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1,2,3,► Major nonsurvivable postoperative complications as described above. Any animal in pain or distress f longer than 72 hours that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain w suspected in the presence of weight loss (10% of body weight), lethargy, limping, vocalizing, excessive licking for over days and even aggression. Wound infection not cured by antibiotics, pneumothorax, hemothorax, suspected severe int bleeding manifesting as hypotension and anemia, respiratory distress symptoms, hypertension.

Surgery 2 and 3 ►In addition to complications described above, unrecoverable nausea leading to significant (>12%) weight loss or no improvement/appetite after 72 hours of anti-emetics/fluid administration.

Surgery 4 and 5- **In addition to complications described above** unrecoverable nausea leading to significant (>12%) weight loss or no improvement/appetite after 72 hours of anti-emetics/fluid administration.

Surgery 6- Terminal surgery

Surgery 7- Major nonsurvivable postoperative complications as described above. Any animal in pain or distress that ca be adequately treated with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of w loss (12% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression. Woun infection not cured by antibiotics, pneumothorax, respiratory distress symptoms, and hypertension.

Surgery 8- 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..

(3) 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]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]0	[redacted]	X	

11. **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	Nerve recording. 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.	**(√)**	**( )**	**( )**	**( )**	**( )**	**( )**	**( )**	**( )**
2	Drug Challenge	(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. The times need to be documented as well so the P.I. can expect when data drop out or potential cross talk occurs. In**

general cleaning occurs in the mornings. 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 in the renal nerve/ganglia and relationship 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:

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.

It has been observed in the bilateral denervated animals (N=2), that after administering clonidine, there is more dramatic hypertension (than in non-denervated animals N=8 or in unilateral denervated animals, N=5), occurring within the first 15 minutes, followed by normalization of BP. We suspect this is due to denervated state. We observed short runs of nonsustained ventricular tachycardia (VT) (<3 sec) during this early period lasting about 15 minutes. We observed it in one animal, but not in another bilateral denervated animal. The observed animal did not experience any ill effects or appear distressed. After 15 minutes, hypertension and ventricular tachycardia subsided. BP recovered to normal (not less than 100mmHg systolic). To combat this, the animal will be monitored for 60 minutes post administration. If sustained VT is observed on the DSI recording for more than 2 minutes, the animal will be administered Amiodarone (7mg/kg IV bolus, every 5 minutes as needed). If hypotension occurs, we will administer fluids. Because this medication is transient, we do not expect sudden cardiac death to occur. We have not observed any evidence of dramatic hypotension as a result of this medication in either two animals.

IV phenylephrine 0.01 mg/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.

[Moise, N., Moon, P. F., Flahive, W. J., Brittain, D., Pride, H., Lewis, B. A., ... & Gilmour, R. F. (1996). Phenylephrine-Induced Ventricular Arrhythmias in Dogs with Inherited Sudden Death. *Journal of cardiovascular electrophysiology*, *7*(3), 217-230.] [Varma S, Circulation Research 1960;8:1182].

The animals will be challenged twice with each of the above drugs. Once approximately two weeks following recovery from survival surgery. The second time will be after induction of PVC-related cardiomyopathy (>2 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.

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 and the development of PVC-induced CM. 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.

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).

2. **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	Cage cleaning will be performed by VMU husbandry staff.	Veterinarian, animal caretakers and protocol staff
2	[redacted]	[redacted]
3		

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	(√)		( )[a]	( )[b]

2	( )		It has been observed in the bilateral denervated animals that after administering clonidine, there is more dramatic hypertension (than in non-denervated animals or in unilateral denervated animals), occurring within the first 15 minutes, followed by normalization of BP. We suspect this is due to denervated state. We observed short runs of nonsustained ventricular tachycardia (VT) (<3 sec) during this early period lasting about 15 minutes. We observed it in one animal, but not in another bilateral denervated animal. The observed animal did not experience any ill effects or appear distressed. After 15 minutes, hypertension and ventricular tachycardia subsided. BP recovered to normal (not less than 100mmHg systolic). To combat this, the animal will be monitored for 60 minutes post administration. If sustained VT is observed on the DSI recording for more than 2 minutes, the animal will be administered Amiodarone (7mg/kg bolus, every 5 minutes as needed). If hypotension occurs, we will administer fluids. Because this medication is transient, we do not expect sudden cardiac death to occur. We have not observed any evidence of dramatic hypotension as a result of this medication in either two animals.	(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)
1					
2	Amiodarone	7mg/kg	Bolus IV	Every 5 minutes as needed	Day of
3					

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

Special Procedure 1 ►

Special Procedure 2 ►The medications given have not been observed to cause any major side effects. As such we have not had to treat it.

Special Procedure 3 ►

Special Procedure 4 ►

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

Special Procedure 1 ►

Special Procedure 2 ►We have not observed any major side effects with IV pharmacologic challenge that require therapy. Generally, if nausea is present secondary to the medications, they resolve within an hour, which is the approximate half life of IV administered drugs. We have observed transient unsustained arrhythmias (PVCs or unsustained VTs lasting <10 beats) that do not require any therapy. If necessary, IV amiodarone 7mg/kg can be given.

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	At the end of study, just before final surgery, telemetry devices will be turned off
2	Present with dog and blood pressure monitoring during procedure and close observation throughout full recovery. The animal will be observed for 60 minutes following administration to combat spontaneous VT.	Drug challenge would not be obtained in those animals with any kind of distress / recent wound or surgical procedures.
3		

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
[redacted]	Richmond, VA 652	American Heart Association funding	Autonomic Nerve Activity and Cardiac Arrhythmias - 02002

	SCORE	DESCRIPTION	ACTION NEEDED BY IACUC
○	0	No concerns noted. Any comments provided are for information only.	*None.* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
●	1	Some concerns noted.	*The IACUC must review the **level 1** 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).* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
○	2	Concerns are noted that must be addressed by the local IACUC and PI before funding can occur, but work described in the ACORP(s) may continue.	*A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. Upload the following at https://vaww.gateway.research.va.gov: (1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed 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).*
○	3	Significant concerns are noted that must be addressed by the local IACUC and PI before funding can occur, and work described in the ACORP(s) listed below must cease immediately.	*A response to each of the **level 3** concerns listed below must be reviewed and cleared by the CVMO* before work can resume and funding can be released. (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.) *A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov : (1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed 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).*

(cont.)

The ACORP for Dr. _____ has received an overall score of 1, which means that it is cleared and represents no bar to funding the application, although some concerns were raised, as shown below.

Please note that a separate score is shown for each of the individual concerns (shown in parentheses under the Item number to which each of the individual concerns refers), to assist you in interpreting the review. An explanation of each of the levels of concern is shown above, in the chart on the previous page. The IACUC must review each of the **level 1** concerns listed 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, but no further correspondence with the CVMO is needed.

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

REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
ACORP (dog)	This ACORP was submitted for triennial review; the ongoing project uses canines in a premature ventricular contraction (PVC) -induced cardiomyopathy model. The project is invasive and challenging to execute. The investigator has taken a "lessons-learned" approach and has modified the procedures accordingly; in particular, the sino-aortic denervation surgical procedure is now performed in two stages (two survival surgeries), which has minimized the risk of gastroparesis. The experimental plan is well-described and includes a clear rationale for the proposed procedures, good attention to animal welfare concerns (nursing care, treadmill training, clear humane endpoints), and supporting literature citations. The comments provided are informational only and are offered for the committee's consideration. An appendix to this review provides additional information for the IACUC's consideration.
Item C.2 (1)	On page 6, the abbreviation EP is used without first defining the term, please clarify. On page 14, the investigator states "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." Please elaborate on the characteristics of each class of heart failure. Also on page 14, the investigator indicates that canine fitness will be based on heart rate and serum lactate acid, please elaborate.
Items D and W (1)	Overall the justification is well-written; please consider also addressing the contribution of *in vitro* studies to this project and why non-mammalian studies are unacceptable. Why are only female dogs used?
Item E (1)	VA Policy requires research training be renewed every two years.

(cont.)

Item U (1)	If an overdose of pentobarbital is used, how is death confirmed?
Item W (1)	The two stage sino-aortic denervation surgical procedure could be listed as a refinement.
Appendix 5 (1)	In regard to the need for wound revision, 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.

Appendix:

652-2002dog20170619 secondary review 11-6-2017
Part of this ACORP would benefit from being rewritten for clarity. Some justifications and explanations could be made stronger.
DETAILED COMMENTS:
Comment 1: General comment: please include a list of abbreviations.

Comment 2: Section B: This section could be difficult for lay readers to understand, and would be stronger if the relevance to veterans' health was pointed out. Try putting something like this at the beginning of the section:
The CDC website says cardiomyopathy affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 10/10/17).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 10/10/17).*
Cardiomyopathy is a condition where the heart muscle does not function well so the heart cannot pump as much blood as it should with each beat. The condition gets progressively worse over time even with the best available treatments, and patients develop symptoms such as shortness of breath, fatigue, swelling in the ankles and legs, irregular heart beat or palpitations, and fainting spells. We think we have a way to stop it from getting worse, which we will test in this study.

Comment 3, Section D (Species justification)
The justification for the species choice could be made clearer and easier for the lay reader to understand.

Try something like this:

For this study we need to deliver PVCs in a controlled fashion, and also have an implanted radiotelemetry device providing real-time data on the ANS activity. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated electronic defibrillator/pacemaker specifically developed for our study. It is approximately 2 inches long, ¼ inch thick and 1.5 inch wide, making it far too large to implant into smaller species such as rabbits or guinea pigs. Although "biological pacemakers" have been developed for smaller species, they cannot be programmed and do not store or analyze data, so they are not suitable for this study. The

(cont.)

radiotelemetry device we need to implant is also too large for smaller species.

Our only options for this work are large animals such as dogs and pigs. Dogs are much more suitable for this work because the canine heart has a His-Purkinje system located in endocardium just like in the human heart. Pigs and other larger animals have a different anatomy, so work with them would not be as relevant to the human condition. Canine heart physiology has also been extensively studied over many years so a lot of information is already available that our study builds upon. Switching to another species such as pigs would require us to start over to some degree, running a lot of pig experiments to reach the point where we already are with dogs before we could even perform this particular study. This process would use many more pigs to get to that point than the 34 dogs required for this study.

Comment 4, section W1 table (literature search). This literature search would be strengthened by doing the following:

1) Change how the search terms are run. Running all the potentially painful or distressing procedures together in a single search means only a paper that includes all of those search terms would be found. Run separate searches instead, since there may be papers that address individual procedures.

2) The database years covered should be at least the last 10 years, not just 2016-2017. There may be papers from the last ten years that are relevant.

In the example below, the first row is a search for unnecessary duplication, focusing on this particular study. The rest are for potentially painful or distressing procedures. Each of these searches brings up less than 30 papers, using the ALTBIB website run by NIH https://toxnet.nlm.nih.gov/altbib.html for the years 2000 – present. (This website works with Google Chrome, but not with Internet Explorer)

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
PubMed	9/15/17	1966-2017	N/A	autonomic nervous system, cardiomyopathy, PVC	( )	( )	( )	(X)
PubMed using ALTBIB animal alternatives search	9/15/17	2000-2017	Cardiac pacemaker implantation	Cardiac pacemaker implantation	(X)	(X)	(X)	( )

(cont.)

strategy								
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	cardiac denervation, stellate ganglion	Cardiac denervation, stellate ganglion	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	cardiac vagal denervation	cardiac vagal denervation	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	PVC challenge	Premature ventricular contraction challenge	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	ventricular programmed stimulation test	ventricular programmed stimulation test	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	clonidine heart test	clonidine heart test	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	9/18/17	2000-2017	phenylephrine blood pressure test	phenylephrine heart blood pressure test	(X)	(X)	(X)	( )

Comment 5, section W2: (Replacement)
This section would be strengthened by including the following information:

a) **Please explain why this research cannot be done with *in vitro* methods (it may be obvious to any biomedical scientist, but this still has to be included).**

(cont.)

b) **The text states that the PI recently described the first canine model of PVC-induced cardiomyopathy in canines. However, another group has described PVC-induced cardiomyopathy in pigs (** https://www.ncbi.nlm.nih.gov/pubmed/26416621 **) Please explain in detail why this work cannot be done in pigs.**

c) **This section has a lot of information that actually belongs in W4 (refinement). Specifically, W4 is where you should put all the things that are done to make the study less painful or distressing to the animals.**

Comment 6, section W3: (Reduction)
The answer provided in this ACORP is unclear. What can be put here instead is:
"We ran a power calculation to determine the smallest number of animals needed for this study. See section C2b for details."

Comment 7, section W4 (refinement)
The term "refinement" confuses a lot of PIs. In this context, it means making the study less distressful or painful for the animals.
Many of the procedures in this study as described in section C2c have refinements which should be noted here in section W4.
Examples are:

1. **How the dogs are gradually trained to run on the treadmill and given treats to do so;**
2. **Training the dogs with treats to calmly sit or lie down for echocardiograms, blood draws, etc.;**
3. **Refining the method for inducing cardiomyopathy so it develops gradually without symptomatic congestive heart failure or signs of distress, etc.**

Please include this information in section W4.

Comment 8, section W5 (lack of unnecessary duplication)
The answer provided in this ACORP is unclear.
Try something like this:
Our literature search for "autonomic nervous system, cardiomyopathy, PVC" yielded only two papers. ***[Then explain how your work differs from or builds on these papers.]***

(cont.)

Literature search 652 Richmond [redacted] 02002 draft 2

1) How is this research relevant to Veterans health?

The CDC website says cardiomyopathy affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 3/10/18).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of long-suffering Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 10/10/17).*

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	3/10/18	All years available	autonomic nervous system, cardiomyopathy, PVC	2

A literature search for "autonomic nervous system, cardiomyopathy, PVC" yielded only two papers.

One was from 32 years ago, and has long since been superseded be subsequent research.

The second paper is from 2017 and uses a pig model.

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].

Small mammals such as rats, mice, guinea pigs and rabbits cannot be used for this project because 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 is too large to use in small animals. The radiotelemetry device is also large and will require internal implantation and observation for several months.

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.

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

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
ALTBIB Citations with Animal Use Alternatives as the main topic	3/10/18	All available years	PVC, cardiomyopathy	0
PubMed	3/10/18	All available years	PVC, cardiomyopathy, computer model	0
PubMed	3/10/18	All available years	PVC, cardiomyopathy, in vitro	4

An ALTBIB search for alternatives to animal use brought up no papers.

No computer models at all were found for this research.

There were four papers on in vitro work:

Two papers looked at drug and hormone effects, and are not useful models what this protocol is studying.

One was about ischemia-induced arrythmias, which is very different from the focus of this protocol.

The last paper was about spontaneous or inducible atrioventricular nodal reentrant tachycardia and not in vitro at all.

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	3/10/18	2000-present	PVC, cardiomyopathy	4
PubMed	3/10/18	All available years	PVC AND cardiomyopathy AND (zebrafish OR invertebrate OR reptile OR bird)	0
PubMed	3/10/18	All available years	PVC AND cardiomyopathy AND sheep	7

An ALTBIB search for animal alternatives using the keywords PVC and cardiomyopathy brought up four papers:

> One was from this group [Huizar 2016], and is actually an in vivo study using the same dog model they will use in this study. The current study will build on the results of the earlier study.
>
> One was about ischemia-induced arrythmias, which is very different from the focus of this study.
>
> One was about spontaneous or inducible atrioventricular nodal reentrant tachycardia and not an in vitro study.
>
> The last paper was about experimental autoimmune myocarditis, which is not a model for PVC-induced cardiomyopathy.

A PubMed search for non-mammalian alternatives (zebrafish OR invertebrate OR reptile OR bird) for studying PVC AND cardiomyopathy also brought up no papers.

Small mammals such as rats, mice, guinea pigs and rabbits cannot be used for this project because 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 is too large to use in small animals. The radiotelemetry device is also large and will require internal implantation and observation for several months.

A PubMed search for the other possible large mammal using the keywords PVC AND cardiomyopathy AND sheep brought up no papers at all.

As noted above, pigs are not as suitable for these studies because 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].

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	thoracotomy	thoracotomy, "cardiac surgery"	5
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	symptomatic congestive heart failure	"symptomatic congestive heart failure", canine	0

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 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.
3) A search on ALTBIB for “symptomatic congestive heart failure, canine” yielded no papers.

<u>Surgery and PVC-induced cardiomyopathy model:</u>

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

The treadmill procedure is designed to minimize distress for the dogs:

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.