University of California, Los Angeles
Chancellor's Animal Research Committee (ARC)

Amendment Application

General Information	
Title:	[redacted]
Protocol #:	[redacted]
PI:	[redacted]
Status:	APPROVED_WITH_CODICIL
Approval Period:	8/9/2019-12/5/2019
Received Date:	8/2/2019
Type:	Amendment
Species:	258 Dog (Pain Category D)
Create Date:	8/2/2019 3:20:04 PM
Created By:	[redacted]
Owner:	[redacted]

Updated Sections
Amendment Summary
Experimental Design
Pain L terature Search
PI Assurance
Proposals
Surgery

Personnel Certifications Due:

[redacted]
- Species Specific Training for Dog

[redacted]
- General Certif cation Test (valid until 2/27/2020)
- Species Specific Training for Dog

[redacted]
- MHQ (val d until 11/27/2019)
- Species Specific Training for Dog

[redacted]
- Species Specific Training for Dog

[redacted]
- MHQ (val d until 11/20/2019)
- Species Specific Training for Dog

[redacted]
- MHQ (val d until 2/19/2020)

[redacted]
- MHQ (val d until 1/17/2020)

Notes:

- General Certif cation Test: Offered through CITI program (**http://www.citiprogram.org**). Please ensure your affiliat on is listed as UCLA and complete the Animal Research Basic Course.
- Medical History Questionnaire (MHQ): Offered by the Occupat onal Health Facil ty (**http://mhq.healthsciences.ucla.edu/**).
- Species Specific Training: Please visit the DLAM webs te: **https://portal.dlam2.ucla.edu/EducationTraining/Pages/default.aspx**.

For more questions regarding certifications/training, please visit:
http://ora.research.ucla.edu/rsawa/arc/pages/certification_info.aspx.

Codicil(s):

- The Committee understands that prior to conducting the next restraint procedure you will conduct a DLAM veterinarian in order to evaluate the animal's response. You may contact DLAM veterinarian Dr. [redacted] to schedule this.

- The Committee understands that all added personnel listed in the Start page will not conduct any procedures with dogs until species specif c training has been conducted and completed by DLAM training/veterinary staff. PLease contact DLAM veterinarian [redacted] in order to schedule this training.

Amendment Summary

Please provide the appropriate information regarding changes to this protocol. Then update the respective sections. If this amendment is requesting a change in personnel, please indicate the individuals you are adding or removing by listing their names in the textbox for question #3 below.

1. Check the following if you will be making any of the following changes:

In addition to checking these boxes, you must update the respective sections of this protocol.

- ☐ Protocol title
- ☑ Funding or funding agency
- ☐ Principal investigator
- ☐ Co-investigator
- ☐ Personnel
- ☐ Location

2. Check the following if you will be making any Significant changes:

In addition to checking these boxes, you must update the respective sections of this protocol.

- ☐ Animal species and/or strain

- ☐ Number of animals
- ☐ Pain category
- ☐ Method of euthanasia
- ☐ Experimental procedures

A. If you indicated that you will be changing the number of animals above, please provide a detailed explanation of your rationale for the number of additional animals requested. Please note that if this request for additional animals also entails a change in experimental procedures and/or pain category, please update these application sections and indicate these changes on this page.

B. If you indicated that you will be changing the experimental procedures above, please provide a detailed explanation of how this change in experimental procedures relates to the experiments in your currently approved protocol. In addition, please clarify what results you hope to yield from this changes in experimental procedures.

3. **In order to assist reviewers, briefly describe in lay terms the changes you are making and complete the appropriate sections. If this amendment is to change funding only, please assure the committee that the research is identical to the previously approved submission. If this amendment is requesting a change in personnel, please indicate the individuals you are adding or removing by listing their names below.**

Adding new funded grant to proposal list.
Adding vagosympathetic trunk to nerve targets for blocking technology

Research Summary

Your answers to the questions on this page determine the other sections needed to be filled out.

1. **What is the Title of the Project?**

[redacted]

2. **Check all that apply:**

- ☐ Tumor Formation (spontaneous or implanted)
- ☑ Chronic Disease (diabetes, EAE, status epilepticus, etc.)
- ☑ Tissue Collection (blood and all other tissues, including those collected after euthanasia)
- ☐ Antibody/Ascites Production
- ☑ Surgical Procedures (survival, non-survival)
- ☑ Non Surgical Procedures (injection of experimental drugs, behavioral studies)
- ☑ Gas Anesthetic Agent(s) (use of isoflurane, halothane, etc)
- ☑ Hazardous Agents (carcinogens, paraformaldehyde, rDNA, vectors, etc.)
- ☐ Radioisotopes or radioactive implants
- ☑ Prolonged Physical Restraint (physical restraint of unanesthetized animals for periods longer than 15 minutes)
- ☐ Genetically Modified Animals
- ☐ Tissue Sharing (use of tissues only)

3. **Will the research be conducted exclusively on tissue received from another investigator?**

No

If yes, do your funding sources require an ARC approved protocol?

No

4. **Check all that apply:**

- ☐ Experiments done entirely at another institution

  NOTE: For experiments conducted entirely at another institut on please subm t the most recent approval not ce and a copy of the most recently approved protocol from the other institution with your submission. Please also indicate the PHS Assurance number and AAALAC accreditation status.

- ☐ Experiments done entirely at VAGLAHS
- ☐ Program Project/Training Grant

  Administrative approval only – no animals associated with this protocol.

- ☐ Breeding Colony: #

  NOTE: If you will be breeding animals for this protocol and do not already have an approved breeding protocol on file w th the ARC, you must submit an Appl cat on to Establish and/or Maintain a Breeding Colony at this time. Check the box above but leave the "Breeding Colony Number" field above empty. The ARC Staff will update the Breeding Colony Number following the submission of a breeding colony application.

5. **If you are seeking approval for a training grant, list all individual projects supported by the program project or training grant, including the principal investigators' names and their current ARC approval numbers. If no animal research is currently being supported by the overall grant, please assure the Committee that, should an investigator of a project covered by the overall grant initiate research involving animals, ARC approval will be obtained prior to the distribution of funds.**

Personnel

There can be only one Principal Investigator per protocol. To edit a person's contact information or add a new person to our system, click on the People tab above.

Prior to the submission of an amendment to add personnel, please ensure that these individuals have completed all applicable animal use certification requirements and have a Medical History Questionnaire (MHQ) on file with the Occupation Health Facility (OHF). If you are only requesting the removal of personnel, please email the ARC administrative office (arc@research.ucla.edu). An amendment application is NOT required if you are only removing personnel.

Principal Investigator

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:		Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Principal Investigator

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is [redacted] has research experience with the canine model going back to [redacted] is a recognized international authority on autonomic control of the normal and diseased heart. Dr. [redacted] has documented expertise in all methods used in this protocol. [redacted] has completed the ARC-approved equivalents of CITI General Certification (Animal Research) and all necessary DLAM training, including species-specific training for dogs.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

As Principal Investigator, Dr. [redacted] will oversee study design and implementation. [redacted] will be involved in all aspects of survival and non-survival surgeries. Additionally, Dr. [redacted] will supervise all data handling, optimization and interpretation. [redacted] will be designated back-up for blood collections from chronic animals via jugular or cephalic vein.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:	[redacted]	Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is board-certified in internal medicine, cardiology and cardiac electrophysiology. [redacted] completed his Ph.D. in the [redacted] in [redacted] and has performed clinical procedures similar to those being performed in this protocol. Dr. [redacted] has experience since [redacted] working with rabbits and swine; [redacted] has completed the CITI General Certification (Animal Research), EHS Laboratory Safety Fundamentals, Hazardous Agent Handling and Waste Management, as well as all required DLAM training, including species-specific training for dogs, pigs, rabbits and rats.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will be involved in all aspects of survival and non-survival surgeries, especially any electrophysiological procedures. [redacted] will be directly involved in blood collection from chronic animals, including from jugular and cephalic vein, with documented experience.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:		**Degree:**	[redacted]
Fax:		**Dept:**	
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is a General Surgery Resident with plans to pursue specialized training in Cardiothoracic Surgery. [redacted] surgical training thus far has included extensive cardiac and vascular surgical experience. [redacted] has a background in investigating cardiopulmonary diffusion capacity, respiratory muscular fatigue and vascular endothelial function in human diabetic patients. Dr. [redacted] has completed the ARC-approved equivalents of CITI General Certification (Animal Research) and all necessary DLAM training, including species-specific training for pigs and aseptic technique.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

[redacted] will be involved in the preparation of the experiments and handling of animals under supervision. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory and preparing the surgical site(s). Additionally, [redacted] will assist the anesthesiologist(s) and surgeon(s) with all aspects of survival and non-survival surgeries in addition to other electrophysiological procedures. Lastly, [redacted] will assist in specimen handling and processing for data analysis.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	[redacted]
Fax:	[redacted]	**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Will this person handle animal tissue in this protocol?

No

Will this person be involved with Survival Surgery Procedures?

No

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is [redacted] will oversee overall [redacted] center aspects of this project.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] is purely supervisory as part of the [redacted] center and will not have direct animal contact during experiments. [redacted] staff [redacted] and [redacted] will provide any animal support under [redacted] direction.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:		**Degree:**	[redacted]
Fax:		**Dept:**	
Status:			

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is an [redacted] that has completed [redacted] cardiology training and is now in [redacted] fellowship program [redacted] is also an incoming [redacted] and will be spending the next 3-4 years working towards [redacted] has completed all the require CITI training classes,including the species specific training for the pig and canine model. [redacted] will be supervised in [redacted] higher level training for canine surgical and anesthetic procedures by Drs. [redacted], [redacted] and [redacted]

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will be involved in electrophysiological data acquisition and analysis. [redacted] will also be involved in high density cardioneural mapping and ablation studies. [redacted] role will also involve preparation of the experiments and handling of animals. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory and preparing the surgical site(s). Lastly, [redacted] will assist the with all aspects of survival and non-survival surgeries in addition to other electrophysiological procedures. As [redacted] gains experience in surgical techniques [redacted] will transition to being a surgical team leader. This transition will be supervised by Drs. [redacted], [redacted] and [redacted]

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	
Fax:		**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

[redacted] is a [redacted] in [redacted] at [redacted] has completed the CITI General Certification (Animal Research), EHS Laboratory Safety Fundamentals, Hazardous Agent Handling and Waste Management, as well as all required DLAM training, including species-specific training for pigs and dogs. [redacted] has previous experience working with swine and will be responsible for helping with both terminal and survival surgeries. [redacted] will work under the guidance of Drs. [redacted], [redacted] and [redacted]

[redacted] has completed all necessary training for survival and nonsurvival surgeries. These include aseptic training.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

[redacted] will be involved in the preparation of the experiments and handling of animals under supervision. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory and preparing the surgical site(s). Additionally, [redacted] will assist the anesthesiologist(s) and surgeon(s) with all aspects of survival and non-survival surgeries in addition to other electrophysiological procedures. Lastly, [redacted] will assist in specimen handling and processing for data analysis.
As [redacted] gains experience [redacted] will be transitioned to primary roles as surgeon and in supervision of animal homeostasis. [redacted] will be trained and supervised in this transition by Drs. [redacted], [redacted] and [redacted]

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	
Fax:		**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

No

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

[redacted] is a highly experienced Staff Research Associate in the DLAM [redacted] has many years of experience working with swine and live-animal surgical preparation, in addition to fluoroscopy instrumentation [redacted] has completed the CITI General Certification (Animal Research) and all required DLAM training, including species-specific training for dogs and pigs.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

[redacted] will be involved in the handling and preparation of the animals. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory, sedation of the animals, intubating/ventilating and preparing the surgical site(s). [redacted] will also assist in

fluoroscopy procedures and will monitor the animals before, during and after transfer to and from the MRI suite for imaging studies.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:		Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is a [redacted] and obtaining [redacted] in [redacted] in [redacted] has been working with small (mouse) and large animal models since [redacted] has completed the CITI General Certification (Animal Research), EHS Laboratory Safety Fundamentals, Hazardous Agent Handling and Waste Management, as well as all required DLAM training, including species-specific training for dogs, pigs, rabbits and mice.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will be involved in all aspects of survival and non-survival surgeries. This entails echocardiogram assessments, setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory and preparing the surgical site(s). Additionally, [redacted] will be involved in all aspects of surgeries (induction, surgery, maintenance and termination). Lastly, [redacted] will assist in specimen handling and processing for data analysis.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

DLAM Staff View Person Detail

Email:		UID:	[redacted]
Phone:	[redacted]	Degree:	
Fax:		Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

DLAM staff will be involved in aspects of survival and non-survival surgery and as such are all trained appropriately for surgical technique and CITI training etc. This includes the post-operative recovery phase.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

They may be involved in obtaining the animal, surgical preparation, anesthesia administration and monitoring if required. The will also be involved in post-operative recovery phases for survival surgery and daily monitoring of the canine animal colony.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:		**Degree:**	[redacted]
Fax:		**Dept:**	
Status:			

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

[redacted] is a [redacted] in the [redacted] and had completed [redacted]. [redacted] has 2 years of clinical research experience at [redacted] Dr. [redacted] has completed the CITI General Certification (Animal Research), EHS Laboratory Safety Fundamentals, Hazardous Agent Handling and Waste Management, as well as all necessary DLAM training, including species-specific training for pigs. [redacted] was trained by Drs. [redacted] and [redacted] in large animal-specific experimentation, including myocardial infarction techniques and open-chest experimentation. [redacted] has experience with the large animal models since since [redacted]

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will be involved in the preparation of the experiments and handling of animals. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory and preparing the surgical site(s). Additionally, [redacted] will assist the anesthesiologist(s) and surgeon(s) with all aspects of survival and non-survival surgeries. [redacted] will assist in specimen handling and processing for data analysis. As Dr. [redacted] obtains experience, [redacted] will transition to primary roles in surgical preparation and supervision of animal homeostasis during experimental procedures. [redacted] will be trained and supervised for this role by Drs. [redacted] and [redacted]

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	[redacted]
Fax:		**Dept:**	[redacted]
Status:	Pol cy 900 Exempt on		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

No

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] has experience since [redacted] in biomedical research. [redacted] is trained as a [redacted] and has multiple peer-reviewed publications in the area of [redacted] has a [redacted] in [redacted] from [redacted] training was completed at the [redacted] in the [redacted] and [redacted] was an independent investigator at [redacted] in the department of [redacted] from [redacted] before joining the [redacted] research group at [redacted] has experience with large animal models since [redacted]

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will harvest nervous tissue at experiment's end prior to animal euthanasia.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	[redacted]
Fax:		**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is board-certified in [redacted] is highly experienced in catheter mapping of [redacted] as electrophysiologic optimization and interpretation. Dr. [redacted] has experience working with large animal models since [redacted] has completed the CITI General Certification (Animal Research), EHS Laboratory Safety Fundamentals, Hazardous Agent Handling and Waste Management, as well as all required DLAM training, including species-specific training for dogs, pigs and rabbits.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Dr. [redacted] will be involved in all aspects of survival and non-survival surgeries, especially any electrophysiological procedures. Dr. [redacted] has [redacted] experience with the canine model and experience large animal models (porcine) since [redacted] is also board certified in [redacted].

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	
Fax:		**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

No

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

[redacted] is a highly experienced Staff Research Associate in the DLAM [redacted] has many years of experience working with live-animal surgical preparation, in addition to fluoroscopy instrumentation. [redacted] has completed the CITI General Certification (Animal Research) and all required DLAM training, including species-specific training for dogs.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

[redacted] will be involved in the handling and preparation of the animals. This entails setting up all instrumentation prior to experiments, assisting in transportation of animals from the vivarium to the laboratory, sedation of the animals, intubating/ventilating and preparing the surgical site(s). [redacted] will also assist in fluoroscopy procedures and will monitor the animals before, during and after transfer to and from the MRI suite for imaging studies.

Will this person handle radioactive materials or radioactive animals?

No

Contacts

Name:	[redacted]
Contact Type:	Emergency
Home Phone:	
Mobile Phone:	[redacted]
Email:	[redacted]

Name:	[redacted]
Contact Type:	Emergency
Home Phone:	
Mobile Phone:	[redacted]
Email:	[redacted]

Funding

1. Funding Types (Check All That Apply):

- [x] Department
- [x] Extramural
- [ ] UCLA Academic Senate
- [ ] Gift
- [ ] No funding at this time
- [ ] Other:

Proposals

List all funding agencies to which this animal protocol has been or will be submitted for consideration. Include all pending applications.

For each grant/proposal subm tted to a funding agency, subm t a copy of the grant proposal. If the agency is not listed, please contact the **Office of Contracts and Grants.** Please note that the National Institutes of Health may be found by typing in the keyword "NIH" when searching for an Agency Code.

Please note that the Publ c Health Service (PHS) Policy requires the Inst tution to verify approval of those components of the grant applicat on or proposal related to the care and use of animals. **Therefore, it is strongly recommended that prior to submission, investigators review all of the proposed experiments pertaining to animals in the grant application to ensure congruence with the animal research protocol. Please detail any inconsistencies between the grant and the protocol in the spaces below.**

Agency Name:

[redacted]

Agency Code:

[redacted]

PI of Proposal/Award:

[redacted]

Proposal/Award Title:

[redacted]

Proposal/Award Number:

[redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

Grant is multi-center grant to define neural networks for cardiac control from mouse to man. Canine model will be used as indicated to define neural circuits responsible for control of regional cardiac electrical and mechanical function. This grant operate under the Other Transactions guidelines. As such, milestones and deliverables are adjusted in consultation with the [redacted] program officer on a quarterly basis. The comprehensive mapping OT2 is charged with defining the structural and functional organization for cardiac control from mouse to man. Animal models are utilized in within the OT2 structure that are appropriate to the question. As such, canine models will be used as appropriate. Funding for the canine experiments will also be made in part using internal funds from the [redacted] and [redacted]

Agency Name:

[redacted]

Agency Code:

[redacted]

PI of Proposal/Award:

[redacted]

Proposal/Award Title:

[redacted]

Proposal/Award Number:

[redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

Species listed in grant is porcine. We will likely evaluate the technologies evolved from this grant in the canine model as well to provide additional pre-clinical validation prior to proof-of-concept studies in humans. This grant operates under the Other Transactions guidelines. As such, milestones and deliverables are adjusted in consultation with the [redacted] program officer on a quarterly basis. The bioelectronic monitoring and control of the heart OT2 is charged with evolving new technologies to transduce and control autonomic projections to the heart using preclinical studies to validate and then transfer to humans. Animal models are utilized that are appropriate to the question. As such, canine models will be used as appropriate, for example to evaluate the efficacy of chronic implants in a chronic setting. Funding for the canine experiments will also be made in part using internal funds from the [redacted] and [redacted]

Agency Name:

[redacted]

Agency Code:

[redacted]

PI of Proposal/Award:

[redacted]

Proposal/Award Title:

[redacted]

Proposal/Award Number:

[redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

No inconsistencies. Proposed studies will evaluate the efficacy of Kilohertz frequency to block nerve traffic in peripheral sympathetic and parasympathetic nerves. Grant specifies canine models as primary test bed.

Rationale

1. Provide a non-technical summary of the overall objectives of the study.

These experiments will provide information on the interactions between the nervous system and heart during the progression of heart disease, specifically after heart attacks. The experiments will provide information on how different parts of the cardiac nervous system and the heart itself can be targeted with either drug therapy or electrical stimulation therapy to slow the progression of heart disease. We will be evaluating the effects of therapies that modify nerve inputs to the heart (neuromodulation) on both electrical and mechanical function of the diseased heart.

2. Indicate the possible benefits to mankind and/or animals or the advancement of knowledge that may be derived from this study.

By learning how the cardiac nervous system responds to the stress of cardiac disease, we can learn new ways to treat that disease process to slow or reverse the progression of heart failure.

3. Explain the rationale for the use of animals, including (a) why the chosen species is the most appropriate for the study and (b) why the chosen species cannot be replaced with a phylogenetically lower species. Note that cost cannot be accepted as a justification.

The dog is ideally suited for these experiments. It has a cardiac nervous system that is similar to humans and there are several experimental models that can be created in the dog that mimic human cardiac disease including ischemic heart disease leading to cardiac cell death {myocardial infarction}, volume overload secondary to mitral regurgitation and congestive heart failure. There is also a substantial body of data which have evaluated the characteristics of cardiac cells, cardiac nerves and structural organization of the dog heart. Moreover, experimental approaches to treat cardiac ischemic heart disease, developed in the dog model, have been effectively transferred to clinical therapy (stem cell implants, adrenergic blocker therapy, etc).

The mandate of translational science is take our knowledge and technologies to the clinical setting. While the porcine model is appropriate for many cardiovascular studies, it is not an autonomically robust model and its form fit of intrathoracic ganglia does not match that of humans (1-9). In the [redacted] we are evolving new technologies to map the structure/function organization of peripheral and central aspects of the cardiac nervous system. The canine model is a well validated model for neural control of the heart for both acute and chronic studies (1, 2, 10). Survival thoracotomies are well tolerated and the temperament of model is well suited to repeat echo imaging studies in the conscious state. The canine model also parallels humans in the primary elements of neural control with regards to structure/function (1, 2, 11). This model has also been approved from our preclinical studies to evolve cardioneural mapping as part of our [redacted] and [redacted] grants.

References

[redacted]

Experimental Design & Justification for Requested Number of Animals

1. Provide a two- to four-sentence lay description of the experimental procedures written in language easily understandable to a seventh grade student.

These experiments will provide information on the interactions between the nervous system and heart during the progression of heart disease, specifically after heart attacks, and during the subsequent development of congestive heart failure. They will provide information on how different parts of the cardiac nervous system and the heart itself can be targeted with either drug therapy or electrical stimulation therapy to slow the progression of heart

disease. We will be evaluating the effects of therapies that modify nerve inputs to the heart (neuromodulation) on both electrical and mechanical function of the diseased heart.

2. Provide a complete description of: (a) all activities involving the use of research animals; (b) a scientific justification for the total number of animals required to conduct this study. The number of animals justified in this section must match the totals in the Pain Category Assignments. To the extent possible, assign all animals to experimental groups, which can be easily distinguished by the independent variables defining each group (e.g.,drug dosages, time points, controls, etc.). Clearly indicate the number of animals needed per group and explain how group sizes were determined, either(i) by statistical analysis, or (ii) where statistics are not applicable (e.g., teaching labs, feasibility studies, antibody production, etc.), on the basis of other considerations (e.g., student/animal ratio, tissue yield per animal, antigen/animal ratio, prior experience, etc.). If statistical analysis is employed to determine the number of animals required, please specify the statistical method used.

Aim 1: To determine the impact of adverse remodeling of the autonomic nervous system (ANS) and the potential for sudden cardiac death in ischemic cardiomyopathy. Ventricular arrhythmias, heart failure, and atrial fibrillation all occur as a result of ischemic heart disease. Understanding how maladaptation's of the ANS, as a result of myocardial ischemia/infarction, alters electrical and neural function at each level of the cardiac hierarchy is critical. Creating a basic anatomic and functional map in an ischemic model will be the foundation of this protocol. There are three different approaches that can be used to create a chronic myocardial infarction. For the 1st approach, using aseptic technique and in an anesthetized state, a catheter will be advanced and used to engage a coronary artery via a femoral arterial sheath. A luminal angioplasty balloon will then be inserted through the catheter into the coronary artery over a coronary wire. The angioplasty balloon will be transiently inflated and microspheres injected into the coronary artery to induce regional myocardial infarction (MI). The catheter is withdrawn and the animals will be monitored daily until termination. Alternatively, chronic myocardial infarction can be created using aseptic technique and via a left thoracotomy. Thereafter, coronary arteries will be isolated (usually the left anterior descending, just caudal to its first diagonal branch) and the vessel occluded in a two-step procedure with a 20 min 50% coronary artery occlusion following by a complete coronary artery occlusion. For these animals, the chest will be closed in layers, residual air withdrawn from the chest cavity and the animals monitored daily until termination. The final approach to create a chronic myocardial infarction is similar to approach 2, but in this approach 3, the total coronary artery occlusion will be released after 90 min. This is a reperfusion model of ischemic heart disease. All other procedures for approach 3 are identical to approach 2. Any animal in which chronic MI is created will undergo only one of the 3 proposed approaches.
The terminal surgical procedure will be 1 week to 6 months after MI creation and will include the manipulations described in the sections below Terminal Procedures (sections 1 to 5) and tissue harvesting (section 7).

Aim 2: To determine the impact of stellate decentralization on neural control of the heart in normal and pathological conditions. Chronic stellate decentralization will be performed in canines within 1-3 weeks post myocardial infarction or in normal canine models (controls). See Surgical Procedures for details of the procedure. In humans, removing the stellate bilaterally reduces ventricular arrhythmias in patients with electrical storm. In animal models, acute stellate resections reduce arrhythmias. These studies will evaluate how chronic stellate decentralization alters processing at all levels of the cardiac neural hierarchy to impact control of cardiac electrical and mechanical function. The terminal procedure will be 1 weeks to 6 months after MI creation (or stellate decentralization). Terminal experiments will include manipulations described in the sections below Terminal Procedures (sections 1-5) and with tissue harvest (section 7).

Aim 3: To determine the impact of targeted bioelectronic stimulation to mitigate adverse remodeling of the autonomic nervous system in the stressed heart. There are 4 different modes of bioelectronic implants that will be used in this aim. For procedure 3A, implant of cardio-defibrillators (ICD's) and pacemakers can be used as a therapeutic option to maintain rhythm control in animal with chronic myocardial infarction. Vagal nerve stimulation (VNS, 3B), spinal cord stimulation (SCS, 3C) or multi-pole cuff electrodes (3D) deployed to the paravertebral chain, ansae subclavia, stellate, or middle cervical ganglia, or vagosympathetic trunk will be used to chronically stimulate and/or block activity of major neural elements of the cardiac nervous system (cervical, spinal cord and intrathoracic). The terminal procedure will be 1 week to 6 months after bioelectronic device implant and will include the manipulations described in the sections below under Terminal Procedures, section 1 to 5, with tissue harvest (section 7). For animals with chronic MI and device therapy, the devices will be deployed at the time of MI creation. For these animals, terminal procedures can include those listed in sections 1-5 with tissue harvest (section 7). The terminal procedure will be 1 week to 6 months after MI creation and/or bioelectronics implant.

3A. Pacemaker or defibrillator implantation: This will involve insertion of a lead into the right ventricle, coronary sinus, left ventricle or right atrium. This is done under aseptic conditions and for survival animals. This will monitor heart rate/rhythm and maintain a heart rate set between specific ranges. This is a chronic therapy to mitigate the potential for ventricular arrhythmias following MI or to correct brady-arrhythmia post-MI.

3B. Vagal nerve electrode and implantable programmable stimulator/generator (IPG): Using aseptic technique and in an anesthetized animal, an implantable electrode will be placed around the cervical vagus nerve to stimulate the parasympathetic nervous system. The lead from the electrode will be tunneled to an IPG positioned in the subcutaneous region of the dorsal neck or back. The IPG can be programmed with an external wand to control various aspects of VNS including on-time, off-time, frequency, pulse width and intensity. As demonstrated in both preclinical and clinical studies, VNS can be safely maintained for years with no discomfort or adverse consequences.

3C. Spinal cord electrode and IPG: This involves inserting a multi-pole electrode via epidural access to stimulate the spinal cord. The lead is then tunneled to an IPG that is placed in a subcutaneous pocket on the dorsal surface at the lower thoracic or upper lumbar region. The IPG can be programmed with an external wand to control various aspects of spinal cord stimulation (SCS) including on-time, off-time, frequency, pulse width and intensity. As demonstrated in both preclinical and clinical studies, SCS can be safely maintained for years with no discomfort or adverse consequences.

3D. Paravertebral/stellate electrode and IPG: This involves insertion of a multi-pole electrode around the sympathetic paravertebral chain in the dorsal thoracic cavity. Alternatively this electrode can be placed around the stellate ganglia, ansae subclavia, middle cervical ganglia or vagosympathetic nerve trunk. The electrode lead is then tunneled to an IPG placed in a subcutaneous pocket on the dorsal surface of the thorax or neck. Alternatively, the electrode lead can be terminated in a transcutaneous button for periodic external stimulation at these same sites. The IPG can be programmed with an external wand to control various aspects of nerve stimulation or bioelectric nerve block including on-time, off-time, frequency, pulse width and intensity.

Aim 4: To evaluate technologies for high density/high through put assessments of autonomic and cardiac function. This aim, supported by the [redacted], [redacted] and [redacted] is to develop and evaluate novel biosensors for neural activity, cardiac electrical function along with probes to detect neurochemicals and other small molecules in the interstitial and vascular spaces. In addition, this aim supports development of new technologies for delivery and assessment of programmed pacing methodologies for arrhythmia identification.

Aim 5: To determine the structural organization of the cardiac nervous system. To determine the location of neurons innervating the heart, and the impact of myocardial infarction on these neurons, injections of retrograde neuronal tracers in the heart will be performed. The animals will undergo myocardial infarction and 4-6 weeks later neural tracer(s) will be injected into cardiac ganglia and/or heart (see surgery section of details of injection). The terminal procedure will take place 1-5 weeks later and will involve harvest of tissues as described in Terminal procedures, section 7. Sham MI animals will serve as controls for these studies.

Terminal procedures for aims 1 to 5:
These procedures will be conducted under continuous anesthesia. Animals will not be allowed to regain consciousness. Justification of animal use (see below) details how animals are distributed among the 5 aims listed above. To minimize animal use, one or more of these procedures may be done concurrently in an individual animal. For example, cardioneural mapping with electrical stimulation of autonomic nerves in conjunction with microdialysis or fast scanning voltammetry placed into the cardiac interstitium.

1. Cardioneural mapping: High density grid electrodes epicardial or endocardial will be used to provide extensive electrophysiological assessment of the beating heart. Flexible penetrating electrodes will also be inserted into atrial and/or ventricular muscle. Flexible penetrating microelectrode arrays will likewise be inserted into one or more of the autonomic or primary sensory ganglia. The cardiac autonomic ganglia included in the neural investigation will include intrinsic cardiac, extracardiac intrathoracic, nodose, and dorsal root ganglion. In addition, following a laminectomy, neural activity may be recorded from penetrating micro-arrays placed over and into the spinal cord from the dorsal surface. One autonomic ganglia or multiple ganglia may be examined individually or simultaneously to minimize animal use.
Objective: to determine the functional control of cardiac function that is mediated by specific nodes of the cardiac nervous system (intrinsic cardiac, intrathoracic and central aspects).

2. Electrical Stimulation of neural structures
a. Sympathetic Ganglia Stimulation: Objective: Electrodes will be inserted into (or around) the paravertebral, stellate, middle cervical or mediastinal ganglia for site specific stimulation of intrathoracic neural structures and to evaluate sympathetic control of cardiac function.
b. Axonal Modulation Therapy: Objective: To determine the effects of axonal conduction stimulation/block (high frequency alternating current or direct current) of intrathoracic nerves (see 2a) on basal and sympathetically-evoked changes in cardiac function.
c. Cervical vagus stimulation (VNS): Objective: Electrode will be wrapped around or inserted into each cervical vagus to assess VNS-induced changes in regional cardio-neural function.
d. Spinal cord stimulation (SCS): Objective: To evaluate the effects of dorsal epidural SCS to impact spinal cord and intrathoracic neural control of regional cardiac function. SCS can be performed via epidural closed access or open laminectomy. Stimuli will be applied to the spinal cord using tonic, burst or high frequency modes.

3. Chemical stimulation of neurites: Objective: To determine the effects of focal chemical stimulation of peripheral ganglia or cardiac sensory neurites on control of regional cardiac function. Chemical neural modifiers will also be infused into the atria, coronary blood vessels, intravenously, or epicardially to activate the cardiac nervous system. Among these are adenosine, angiotensin II, atropine, atenolol, bradykinin, Capsaicin, hexamethonium, ivabradine, isoproterenol, metoprolol, nicotine, norepinepherine, epinephrine, phenylepherine, resinferatoxin, substance P, Veratridine. See medications section for doses and routes of administration.
For these chemicals, pharmaceutical grade drugs will be used when available. If not, solutions will be sterilized through a 0.22um filter prior to use.

4. Cardiac autonomic stressors: Objective: To determine interdependent cardioneural responses to focal activation of cardiovascular afferents. Stressors will include physical touch of the heart and transient occlusion of the inferior vena cava, descending aorta and/or coronary arteries. Cardiac stressors can be done directly and/or percutaneously. Stressor will also include point specific cardiac pacing delivered to the cardiac surface or within heart muscle. Baseline and evoked changes in cardio-neural function will be evaluated (see 4b) as well as indices of the interstitial fluid environment including levels of neurotransmitters (see 4a).

4a. Neurochemical markers: Microdialysis catheters will be inserted into the myocardium to obtain periodic samples of interstitial fluid. Catheters will be perfused with precision infusion pumps and samples collected.
Alternatively, wires (usually PFA-coated platinum wire with sizes ranging from 50 to 150 µm) will be inserted into the myocardium or vascular compartment and/or plunge electrodes will be inserted into cardiac and neural tissues. From these sensors the cardiac microenvironment will be assessed by fast scanning (low current) voltammetry or capacitative immuno-sensors.
4b. Cardioneural markers: see above. Neural markers will include direct recording of autonomic nerve activity. Cardiac markers will include insertion of transducers to record indices of cardiac electrical and mechanical function.

5. Autonomic denervation: Objective: To evaluate neural control of the heart by a loss of function approach. Autonomic disruption of cardiac control can include chemical (phenol, local anesthetics, ganglionic blockers), surgical or ablative denervation of intrinsic cardiac ganglia, stellate/middle cervical ganglion, cervical vagus transection or spinal cord root transection.

6. Termination: the animal will be sacrificed using pentobarbital + potassium chloride or VF induction with low voltage (~9 volts) direct current under inhaled isoflurane 5%.

7. Tissue collection: Objective: To evaluate structural and neurochemical changes in the canine cardiac nervous system; normal vs cardiac pathology states (with and without bioelectronic therapies - see aim 3 above). The following tissues will be explanted for further studies: heart (including intrinsic cardiac ganglia), mediastinal ganglia, stellate ganglia, paravertebral chain, vagus nerves, middle cervical ganglia, nodose ganglia, spinal cord and dorsal root ganglia for histopathology or metabolic analysis. Nervous tissue may be harvested just prior to euthanasia and under surgical plane anesthesia. Following euthanasia, additional samples make by taken from the central nervous system or viscera to assess multi-organ impact of cardiovascular disease.

Justification of sample size

Group size of 10 was calculated using an α (two-tailed) of 0.05 (type 1 error rate), β of 0.250 (type 2 error rate), an E (effect size) of 0.500 and a standard deviation of change in outcome of 0.600.
Total n=258 over 3 years. For projects 1,2, 3 and 5 group size is 10 for normal animals and increased to 12 for chronic myocardial infarction. The increase in the chronic MI group is necessitated by the expected 20% loss of animals to MI induction, primarily due to ventricular fibrillation. For aim 5, we have minimized animal use by harvesting tissues from multiple levels of the cardiac nervous system in response to each cardiac injection site.

Definitions:
ICN=intrinsic cardiac nervous system; MCG=Middle Ganglia; KHFAC=kilohertz high frequency alternating current; DC=direct current; VNS= vagus nerve stimulation.

Aim 1: Cardioneural mapping with stressors: (n=44 aim 1)
a. ICN: normal, n=10; chronic MI n=12
b. Stellate/MCG: normal, n=10; chronic MI n=12
Aim 2: Stellate Decentralization (n=22 for aim 2)
a. Normal (Sham), n=10
b. Chronic myocardial infarction, n=12
Aim 3: Bioelectronic implants (n=88 for aim 3)
a. VNS
1) Normal (sham), n=10
2) Chronic myocardial infarction, n=12
b. Spinal cord stimulation
1) Normal (sham), n=10
2) Chronic myocardial infarction, n=12
c. KHFAC electrode, paravertebral chain
1) Normal (sham), n=10
2) Chronic myocardial infarction, n=12
d. DC electrode, paravertebral chain
1) Normal (sham), n=10
2) Chronic myocardial infarction, n=12
Aim 4: Technology assessments for bioelectronic monitoring of the heart (n=60 for aim 4)
a. 20 animals/year for (requesting 60 as part of this approval approval cycle
b. To minimize animal use, animals will be multi-tasked for evaluation of the neural sensors, cardiac electrical sensor and small molecule detectors.
Aim 5: Neural track tracing (n=30 for aim 5) (note for aim 5 10 animals will be normal and 24 animals will have tracer injections done 4-6 weeks after chronic myocardial infarction induction). We expect 20% loss in chronic MI model so number of animals per injection site is increased from 5 to 6.
Normals (n=20 for aim 5)
a. Atrium to different levels of cardiac nervous system (ICN, stellate/MCG ganglia, nodose and DRG); n=5 for right atrial injections; n=5 for left atrial injections.
b. Ventricle to different levels of cardiac nervous system (ICN, stellate/MCG ganglia, nodose and DRG), n=5 for right ventricular injections, n=5 for left atrial injections.
Chronic MI (n=24 for aim 5)
a. Atrium to different levels of cardiac nervous system (ICN, stellate/MCG ganglia, nodose and DRG); n=6 for right atrial injections; n=6 for left atrial injections.
b. Ventricle to different levels of cardiac nervous system (ICN, stellate/MCG ganglia, nodose and DRG), n=6 for right ventricular injections, n=6 for left atrial injections.

The animals will be acquired from an approved vendor which is currently [redacted] All medications will be pharmaceutical (or Veterinary) grade with the following exceptions.
Alpha-chlorolase - Chemical grade
Cholera Toxin - Chemical grade (for track tracing)
Dil - Chemical grade (for track tracing)
Fluoro - Gold - Chemical grade (for track tracing)
Nicotine - Chemical grade (for stimulating autonomic ganglia in acute preparations)
TMR-D - Chemical grade (for track tracing)
Wheat germ agglutinin - chemical grade (for track tracing)
These agents will be passed through 0.2um filter prior to use.

Pain Category Assignments

NOTE: A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to wh ch that procedure is applied. Examples of potentially painful/distressful procedures include, but are not lim ted to the following: terminal surgery; exuberant inflammation from adjuvants; ocular and skin irritancy testing; food or water deprivation beyond that necessary for normal presurgical preparation; noxious electrical shock that is not immediately escapable; paralysis or immobility in a consc ous animal; extensive irradiation.

Category	Description
C	Momentary or no pain/distress (Examples: injections of non-toxic substances; peripheral blood collections not requiring anesthesia; euthanasia and harvesting of tissue only; observing natural behavior; behavioral testing without signif cant restraint or noxious stimuli.)
D	Pain/distress relieved by use of appropriate anesthetics, analgesics, tranquilizers or by euthanasia (Examples: terminal surgery; survival surgery; retro-orbital blood collection; euthanasia of animals showing signs of more than slight or momentary pain and/or distress.)
E	Pain/distress can not be relieved by use of anesthetics, analgesics, or tranquilizers, as the use of these agents would interfere w th the experimental design (Examples: pain research; toxic ty testing.)

Species:	Dog
Strain or Breed (if applicable):	mongrel
Average Weight:	20-30 kg
Sex:	Mixed
Pain Category:	D
Previous Number of Animals Approved:	258
Change in Number of Animals Needed (+/-):	0
Number of Animals Needed for the 3 Year Period:	258

Pain Category

1. If the animals are listed under Pain Category D and/or E, check below all criteria that will be used to assess any potential pain/distress/discomfort in the animals. If applicable, include criteria used to evaluate post-operative pain/discomfort.

- [x] Restlessness
- [x] Vocalizing
- [x] Decreased or impaired mobility
- [ ] Conjunctivitis, corneal edema, photophobia
- [x] Licking, biting, or guarding a painful area
- [ ] Failure to groom, unkempt appearance
- [x] Open sores/necrotic skin lesions
- [x] Loss of appetite
- [x] Weight loss.
  Percentage weight loss (max allowable 20%): 10%
- [x] Other: Difficult, exaggerated or abnormally fast breathing pattern

2. If the animals are listed under Pain Category E, please specify the pain/distress/discomfort experienced by animals as a result of the experimental manipulations and provide scientific justification indicating why pain/distress/discomfort-relieving methods will not be employed in this protocol.

NOTE: Procedures that may cause more than momentary or slight pain or distress to the animals must be performed with appropriate sedatives, analgesics or anesthet cs, unless withholding such agents is justified for scientific reasons and will continue for only the necessary per od of time.

NA

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E. Federal Regulations require that investigators consider alternatives (the 3 Rs - replacement, refinement and reduction) to procedures that may cause more than momentary or slight pain or distress to animals.

3. Consider all the alternatives listed below and explain why each of the following is not an available alternative for the proposed potentially painful/distressful procedure.

A. Replacement of animals with non-animal models (e.g., in vitro procedures, computer model) or a phylogenetically lower species:

The dog is ideally suited for these experiments. It has a cardiac nervous system that is similar to humans and there are several experimental models that can be created in the dog that mimic human cardiac disease including ischemic heart disease leading to cardiac cell death {myocardial infarction}, volume overload secondary to mitral regurgitation and congestive heart failure. There is also a substantial body of data which have evaluated the characteristics of cardiac cells, cardiac nerves and structural organization of the dog heart. Moreover, experimental approaches to treat cardiac ischemic heart disease, developed in the dog model, have been effectively transferred to clinical therapy (stem cell implants, adrenergic blocker therapy, etc).

These studies require direct cardiac interventions given the goals of the study. As such, it is critical to assess parameters of cardiac disease (our main focus) using a live animal model to further understand their underlying mechanisms as would relate to the clinical field. Data collection via non-surgical approaches such as computer simulations is not feasible for this study as it may contain parameters essential but unknown to the genesis of cardiac dysfunction. Therefore, this method may not be the most viable option for simulating cardiac abnormalities as observed with in-vivo studies.
Canines can't be replaced by lower animals because phylogenetically lower species do not have autonomic ganglia that are of sufficient size to allow for microarray in vivo recording. Moreover, peripheral autonomic ganglia of rats and rabbits do not demonstrate the phenotype diversity of larger animals. In addition canines can't be replaced by a porcine model as for any cardiac study that requires a high degree of interaction with Pavlov stands, echocardiography, device interrogation/adjustment is better suited to a canine model.

B. Please discuss why the procedures cannot be further refined in order to minimize potential pain and/or distress to animals:

These studies require direct cardiac interventions given the goals of the study. As such, it is critical to assess parameters of cardiac disease (our main focus) using a live animal model to further understand their underlying mechanisms as would relate to the clinical field. Data collection via non-surgical approaches such as computer simulations is not feasible for this study as it may contain parameters essential but unknown to the genesis of cardiac dysfunction. Therefore, this method may not be the most viable option for simulating cardiac abnormalities as observed with in-vivo studies.

C. Reduction in the number of animals proposed in this application (e.g., fewer animals involved in potentially painful procedures):

Using Student's T test, for a 90% power with α=0.05, the sample size is calculated as below in summary of animal justification. Total n=244 over 3 years. For projects 1,2,3 and 5, group size is 10 for normal animals and increased to 12 for chronic myocardial infarction. The increase in the chronic MI group is necessitated by the expected 20% loss of animals to MI induction, primarily due to ventricular fibrillation. For aim 5, we have minimized animal use by harvesting tissues from multiple levels of the cardiac nervous system in response to each cardiac injection site. For aim 4, we have minimized animal use by evaluating multiple technologies within the same animal model (neural interfaces, cardiac electrical interfaces and chemical sensor probes).

Pain Literature Search

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E.

Please note that according to PHS Policy IV.C.1.a, the Guide for the Care and Use of Laboratory Animals (the Guide p. 10) and USDA Animal Welfare Act Regulat ons §2.31(d)(1)(i) "procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals." Further, in order to meet the above-ment oned regulatory requirement and in accordance with UCLA's Animal Welfare Assurance on file w th the National Institutes of Health Off ce of Laboratory Animal Welfare (OLAW), the Committee must ensure that the "principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has prov ded a wr tten narrative descript on of the methods and sources used to determine alternatives were not available." Please also note that the Comm ttee recommends the use of keywords that are specif c to the painful/distressful procedures you will be conducting and the animal model that will be used.

1. Indicate at least two databases or other sources consulted to support the conclusion that appropriate alternatives are not available.

- [x] Pubmed (Medline)
- [ ] PsychINFO
- [ ] Altweb
- [x] UC Center for Alternatives
- [x] Animal Welfare Information Center
- [ ] BIOSIS
- [ ] Current Contents
- [ ] Other:

2. Combination of keywords used during the search:

Please specify the keywords used in the box below, including 1) the specific painful procedures that you are conducting, 2) the animal model being used and 3) alternative terms (e.g., animal model, welfare, pain, stress, distress, methods, *in vitro*).

Please see the following examples, noting that the keywords listed only apply to a protocol involving these experimental variables:

```
Mouse and chronic implant and in vitro model
Mouse and artery ligation and pain
Mouse and sleep deprivation and welfare
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Keywords used:

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Intrinsic cardiac neurons and sympathetic nervous system and canine
Intrinsic cardiac neurons and parasympathetic nervous system and canine
Intrinsic cardiac neurons and myocardial ischemia and canine and distress
Sympathetic neurons and myocardial ischemia and canine and distress
Vagal nerve stimulation and myocardial ischemia and canine and distress
Vagal nerve stimulation and heart failure and canine and distress
spinal cord stimulation and cardiac nervous system and canine
stellate stimulation and cardiac nervous system and canine and distress
Kilohertz AC nerve block and sympathetic nervous system and canine and distress
DC nerve block and sympathetic nervous system and canine and distress
Kilohertz AC nerve block and parasympathetic nervous system and canine and distress
DC nerve block and parasympathetic nervous system and canine and distress
Additional search:
Pain control
Congestive heart failure
Dog
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3. Date of most recent search (MM/DD/YYYY):

NOTE: The l terature search must be updated whenever experiments that may cause potential pain or distress are proposed/modified. The literature search must also be updated at the time of each three-year renewal, and should be conducted within 2 months of submission.

8/2/2019

4. Years Covered (e.g., 1980-2019):

1966 - present

Animal Care

1. Will the experiments involve tumor formation?

The ARC requires daily monitoring of tumor growth.

No

2. Will the experiments involve chronic disease (e.g., diabetes, chronic seizures, infections with disease agents) or a chronic condition (e.g. headcaps, implants)?

Yes

3. Will the experiments involve other procedures that may lead to potential complications (e.g., surgical procedures, administration of compounds with potential toxic effects)?

Yes

4. For <u>all</u> types of experiments, if animals may experience complications, please describe the criteria for premature euthanasia below.

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On the day of surgery, animals are checked during the evening (approximately between 6-9 PM). Monitoring includes
visual examination of general appearance, respiratory rate, blood pressure, heart rate, temperature and
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membrane color, incision site, and behavior. Clinical signs used to indicate discomfort include the following symptoms: restlessness, increased respiratory rate and/or increased respiratory effort, vocalization, increased blood pressure, increased heart rate, lack of mobility, licking/biting/scratching of the incision site.
For survival surgery, analgesia and antibiotic therapy is provided as detailed in Medications section.
Animals are monitored 2x daily by lab authorized personnel and/or DLAM personnel for 1 week post-op and then daily thereafter.

Other supportive care:
Vitamin B12 (1500-3000mcg, IM, SQ) ¬- It is given post-operatively if needed to stimulate the appetite, and is repeated as needed.
Lactated Ringers Solution (30ml/lb/day SQ or iv) - Fluids are given if the animal is not drinking or appears dehydrated (i.e. - increased skin turgor, tenting of skin, rough coat, weight loss, sunken eyes).
Diet - In addition to their maintenance diet, animals may be offered canned food, dog bones, etc. as treats.
Monitoring will include visual examination of general appearance, healing of the incision, respiratory rate, mucous membrane color, heart rate, blood pressure, temperature, appetite and behavior. Body weight will be recorded at least once a week for the week post-op. Sutures will be removed 10-14 day post-op.
Animals will be monitored daily by one of the laboratory authorized personnel or by special arrangement with the DLAM staff.

Criteria for euthanasia are the following: mutilation of the surgery site not easily repaired, infection not resolved with antimicrobial therapy, moderate to severe clinical signs of pain and distress un-alleviated by appropriate analgesics, overt clinical signs of late heart failure (Dyspnea, excessive pulmonary edema not resolved with diuretic therapy, and failure to stand with stimulation).
Plan for dealing with anticipated complications:

Hemorrhage: The chance for blood loss is minimal with all surgical techniques discussed in this protocol. Blood loss associated with the procedure can be effectively controlled with suture ties and pressure. In the unusual condition where blood loss is severe, the animal will be terminated.

Infection: Infections associated with the incision sites will be treated with appropriate antibiotic therapy as determined in consultation with DLAM DVM.

Mutilation of the surgical site: If the mutilation is minor (skin only), then the site will be cleaned at least twice daily with betadine solution and antibiotic therapy initiated. If needed, a bandage and protective jacket will be placed on the chest. If necessary an Elizabethan collar can be placed on the animal.

Loss of appetite and weight loss: Animals are given vitamin B12 post-op to stimulate the appetite. If the animal is not drinking, fluids (SQ or iv) are given.

Pneumothorax: Residual air is removed during the procedure and the lungs are purged prior to removing the chest tube. Any residual air should be resorbed. If a pneumothorax is suspected and the animal shows signs of distress, then the animal's chest will be prepped with betadine and a 20 gauge needle catheter will be inserted into the chest cavity and residual air withdrawn. The animal may also be terminated if distress is severe.

Pulmonary Congestion: Hearts and lungs are auscultated on a daily basis. If animals exhibit onset of pulmonary venous congestion, furosemide will be given at as needed and in consultation with DLAM veterinary staff.

5. Check below all that apply to convey special animal care requirements to the responsible veterinary staff.

- ☐ Temperature Range(s)
- ☐ Humidity
- ☐ Light Cycles
- ☐ Bedding/Litter changing schedules
- ☐ Water (e.g., sterile or deionized)
- ☑ Special diet/Feeding schedule
- ☐ Deprivation of food and/or water for reasons other than surgical preparation

6. If you checked any of the boxes above, explain special care requirements in detail.

Treats should be restricted to about 0.3% sodium.

7. Environmental Enrichment: [redacted] vivarium staff provide environmental enrichment to all species (please refer to the **ARC Policy on Environmental Enrichment**).

a. If you request to provide additional or alternative environmental enrichment, please describe the environmental enrichment below.

Before surgery, animals may be grouped housed.

b. Please provide scientific justification if your research precludes the use of environmental enrichment.

Animals should be housed individually for 7-14 days post-op to avoid incisional trauma from other dogs. Dogs in overt heart failure that appear distressed or bothered by other dogs should also be housed alone. This is because social stress induces another level of sympathetic stimulation. Such "excitement" can cause a transition from compensated to decompensated heart failure.

To facilitate animal socialization, cages will be adjusted as possible to allow for visual and "nose-to-nose" contact between adjacent animals. Laboratory staff will also interact with the animals as part of the daily "rounding" on our chronic animal population.

8. If you will be using transgenic animals in this research, please clarify whether there are any anticipated or suspected phenotypes of the transgenic mice that might cause pain or discomfort to the animals. If any pain, distress, or morbidity is associated with the phenotypes of this line, please indicate the criteria for premature termination of these mice.

NA

9. PLEASE COMPLETE IF YOU HAVE MICE AND/OR RATS IN DLAM-MANAGED FACILITIES. Please check one response to the following:

I request that the veterinarian (or his/her designee) euthanize animals found to be sick or injured for me:

○ I request that the DLAM veterinarian (or his/her designee) euthanize my animals for me in accordance with his/her veterinary discretion at the time that they are found sick or injured. This decision will only apply to animals in cages that I've marked with a green euthanasia sticker on the cage card. DLAM will notify me of the euthanasia by email after the fact.

I understand that I remain responsible for monitoring of my animals, in accordance with my approved protocol and with the ARC Policy on ***Responsibility for Monitoring Laboratory Animals***.

I will treat or euthanize animals:

○ I assure the ARC that I will promptly respond to Veterinary Health Case notifications regarding my animals, as required by the ARC Policy on ***Notification of Investigators with Sick or Injured Animals***.

Locations

Please indicate ALL locations where animals will be housed and/or used, including:

1. **Vivarium Housing** (where animals will be housed). Please note that if vivarium housing has not been assigned, select "VIVARIUM" as the building name and "Unassigned" as the room number.
2. **Study Area** (any investigator-maintained facility outside the vivarium where USDA-covered species will be housed for per ods longer than 12 hours, or where non-USDA-covered species will be housed for per ods longer than 24 hours).
3. **Research Area** (where non-surg cal activities, including euthanasia, will be performed).
4. **Surgery Area - Survival** (where recovery surgery will be performed).
5. **Surgery Area - Non-Survival** (where terminal surgery will be performed).

Building	Room	Species	Location Type
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** Terminal surg cal procedures. This rooms will function as the primary research focal point for our [redacted] As such there will be multiple procedures done in this room on a weekly basis. Our operat onal capacity is increasing an order of magnitude. These rooms will support multiple projects funded by the [redacted] and private industry. They are equipped w th state of the art equipment for concurrent cardiac electrophysiology and neural mapping. These systems are un que to our operation and not available in your current facilities. They are not pieces of equipment that can be moved in and out of a shared access room.
[redacted]	[redacted]	Dog	Research Area **Reason:** MRI
[redacted]	[redacted]	Dog	Surgery Area - Survival
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** Terminal surg cal procedures. These two rooms ([redacted]) will function as the primary research focal point for our [redacted] As such there will be multiple procedures done in both rooms on a weekly basis. Our operational capac ty is increasing an order of magnitude. These rooms will support multiple projects funded by the [redacted] and private industry. They are equipped with state of the art equipment for concurrent cardiac electrophysiology and neural mapping. These systems are un que to our operation and not available in your current facilities. They are not pieces of equipment that can be moved in and out of a shared access room.
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** Terminal surg cal procedures.
[redacted]	[redacted]	Dog	Research Area **Reason:** Room will be used for echocardiography, pavlov stand procedures including baseline electrograms and vagal nerve stimulations. It will also be used to setup holter recordings and if necessary for euthanasia for animals not undergoing terminal procedures.
[redacted]	[redacted]	Dog	Vivarium Housing
[redacted]	[redacted]	Dog	Vivarium Housing
[redacted]	[redacted]	Dog	Vivarium Housing
[redacted]	[redacted]	Dog	Vivarium Housing
[redacted]	[redacted]	Dog	Research Area **Reason:** Advanced imaging of CNS and heart.

Medications and Experimental Drugs

List below all medications/drugs/compounds/agents/etc. that will be given to the animals. Please be sure to include analgesics, anesthetics, antibiotics and all experimental drugs or treatments. Cell lines injected in suspension should be listed here.

The select on of the most appropriate med cation/agent should reflect that which best meets clin cal and humane requirements w thout compromising the scientific aspects of the research protocol. In accordance w th federal regulat ons, consultat on with an attending veterinarian is required in the planning of a research protocol involving procedures that may cause more than momentary or slight pain or distress to the animals. The **ARC Policy on Use of Pharmaceutical-Grade Compounds** requires that investigators use pharmaceut cal-grade compounds whenever they are available, even in acute procedures.

If pharmaceut cal-grade preparations are not available, please dentify wh ch compounds are affected and provide supporting justification in your Experimental Design. All non-pharmaceutical-grade drugs must be filter-sterilized pr or to use.

Please do not list euthanasia drugs in this section.

Drug/Compound Name:	Buprenorphine
Species:	Dog
Medication Type:	Analges c

Dose or Concentration:	0.005 - 0.03 mg/kg
Volume:	Dependent on weight
Frequency:	6-8 hours
Route:	im
Length of treatment/administration:	Pre-op, as needed post-op at 6-12 hr intervatls
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Buprenorphine SR
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	0.03-0.06 mg/kg
Volume:	dependent upon weight
Frequency:	1
Route:	im
Length of treatment/administration:	72 hours durat on, repeated if indicated
Purpose:	Post-Operative

Drug/Compound Name:	Carprofen (Rimadyl)
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	3.0 - 5.0 mg/kg
Volume:	Tablet
Frequency:	2x daily
Route:	other: SC, IM or PO
Length of treatment/administration:	As needed post-op
Purpose:	Post-Operative

Drug/Compound Name:	Fentanyl
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	10 -30 mcg/kg/hr
Volume:	Dependent on weight
Frequency:	Single administration
Route:	iv
Length of treatment/administration:	2 hrs
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Marcaine
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	1 mg/kg
Volume:	2 ml
Frequency:	1
Route:	sc
Length of treatment/administration:	1 hr
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Meloxicam
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	0.2 mg/kg
Volume:	2 ml
Frequency:	pre-op, 1x day for 7 days post-op
Route:	other: SC or PO
Length of treatment/administration:	7 days post-op
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Alpha-chloralose [redacted]
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	50 mg/kg/hr
Volume:	Dependent on weight
Frequency:	Continuous infusion
Route:	iv
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Post-Operative Other: Anesthest c following surg cal preparat on

Drug/Compound Name:	Etom date
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	0.5-4 mg/kg
Volume:	dependent upon weight
Frequency:	1
Route:	iv
Length of treatment/administration:	duration of surgical procedure
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Isoflurane
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	1 - 3%
Volume:	n/a
Frequency:	Single, continuous infusion
Route:	inh
Length of treatment/administration:	Single, continuous administration
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pentobarb tal
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	100-200 mg/kg
Volume:	1-5 ml
Frequency:	1
Route:	iv
Length of treatment/administration:	1-5 min
Purpose:	Pre-Operative/Intra-Operative Other: terminat on

Drug/Compound Name:	Propofol
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	3-8mg/kg
Volume:	Dependent on weight
Frequency:	25% of dose every 30 seconds till intubation
Route:	iv
Length of treatment/administration:	Single administration
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Cefazolin
Species:	Dog
Medication Type:	Antib otic
Dose or Concentration:	7 -10 mg/kg
Volume:	oral
Frequency:	1x daily
Route:	oral
Length of treatment/administration:	7 days and as needed thereafter
Purpose:	Post-Operative

Drug/Compound Name:	Cefovecin
Species:	Dog
Medication Type:	Antib otic
Dose or Concentration:	8 mg/kg
Volume:	2 ml
Frequency:	1
Route:	sc
Length of treatment/administration:	7 day duration, as indicated thereafter
Purpose:	Post-Operative

Drug/Compound Name:	Cepazolin/Cephalexin
Species:	Dog
Medication Type:	Antib otic
Dose or Concentration:	22 - 30 mg/kg
Volume:	Tablet, IV dependent on weight

Frequency:	2x daily
Route:	other: PO or IV
Length of treatment/administration:	7 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	epinephrine
Species:	Dog
Medication Type:	Antib otic
Dose or Concentration:	.1-.2 mg
Volume:	1 ml
Frequency:	as needed
Route:	iv
Length of treatment/administration:	as needed
Purpose:	Post-Operative

Drug/Compound Name:	amiodarone
Species:	Dog
Medication Type:	Other
Dose or Concentration:	5-9 mg/kg
Volume:	2 ml
Frequency:	as needed
Route:	iv
Length of treatment/administration:	as needed
Purpose:	Non-Surgical Procedures

Drug/Compound Name:	Ang otensin II
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 uM
Volume:	1 mL
Frequency:	1-4x
Route:	other: iv or topical
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Pre-Operative/Intra-Operative Other: Cardiac stressor

Drug/Compound Name:	Atenolol
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 - 2 mg/kg
Volume:	1 mL
Frequency:	2-5x
Route:	iv
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Atropine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	.05-3 mg/kg
Volume:	10 mL
Frequency:	2x daily
Route:	iv
Length of treatment/administration:	1 day
Purpose:	Other: Evaluate parasympathetic function

Drug/Compound Name:	benzodiazepine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.25- 1 mg/kg
Volume:	dependent upon weight
Frequency:	1
Route:	iv
Length of treatment/administration:	duration of surgical procedure
Purpose:	Other: reduce vomiting and tremors associated with etom date

Drug/Compound Name:	Cholera Toxin subunit B conjugated with fluorophore
Species:	Dog
Medication Type:	Other
Dose or Concentration:	.05-1%
Volume:	100-1000 ul
Frequency:	1 to 20
Route:	other: direct inject on ganglia, nerves, heart
Length of treatment/administration:	30 min to complete inject ons
Purpose:	Other: tract tracing for cardiac nervous system

Drug/Compound Name:	DiI
Species:	Dog
Medication Type:	Other
Dose or Concentration:	5-10 mg/ml
Volume:	100-1000 ul
Frequency:	1 to 20
Route:	other: direct inject on ganglia, nerves, heart
Length of treatment/administration:	30 min to complete inject ons
Purpose:	Other: tract tracing for cardiac nervous system

Drug/Compound Name:	Enalapril
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.25 - 1.00 mg/kg
Volume:	Tablet
Frequency:	2x daily
Route:	oral
Length of treatment/administration:	2 - 4 wks
Purpose:	Other: ACE inhib tor

Drug/Compound Name:	Esmolol
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 - 2.0 mg/kg
Volume:	Dependent on weight
Frequency:	Once, as needed
Route:	iv
Length of treatment/administration:	Premedicat on, single administration
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	famot dine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	2 mg/kg
Volume:	2 ml
Frequency:	as needed
Route:	iv
Length of treatment/administration:	as needed
Purpose:	Post-Operative

Drug/Compound Name:	Fluoro-Gold
Species:	Dog
Medication Type:	Other
Dose or Concentration:	2 mg/ml
Volume:	100-1000 ul
Frequency:	1 to 20
Route:	other: direct inject on ganglia, nerves, heart
Length of treatment/administration:	30 min to complete inject ons
Purpose:	Other: tract tracing for cardiac nervous system

Drug/Compound Name:	Furosemide
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 - 4.4 mg/kg
Volume:	Tablet, IM/IV dependent on weight
Frequency:	1-4 times daily
Route:	other: PO, IM or IV
Length of treatment/administration:	As needed post-op

Purpose:	Post-Operative

Drug/Compound Name:	furosem de
Species:	Dog
Medication Type:	Other
Dose or Concentration:	2-4 mg/kg
Volume:	
Frequency:	as needed
Route:	other: PO or im
Length of treatment/administration:	as needed
Purpose:	Post-Operative

Drug/Compound Name:	Glycopyrrolate
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.1 - 0.5 mg
Volume:	3 mL
Frequency:	2x daily
Route:	iv
Length of treatment/administration:	2 hrs
Purpose:	Other: Evaluate parasympathetic function

Drug/Compound Name:	Heparin
Species:	Dog
Medication Type:	Other
Dose or Concentration:	150 - 300 un ts/kg
Volume:	1.0 - 10 mL
Frequency:	Once, as needed
Route:	iv
Length of treatment/administration:	Single administration
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Ivabradine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 to 5.0 mg
Volume:	Tablet
Frequency:	2x daily
Route:	oral
Length of treatment/administration:	2-4 wks
Purpose:	Other: Funny channel blocker

Drug/Compound Name:	Ketamine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	2.5-7.5 mg/kg
Volume:	dependent upon weight
Frequency:	1
Route:	iv
Length of treatment/administration:	duration of surgical procedure
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Lactated Ringers
Species:	Dog
Medication Type:	Other
Dose or Concentration:	30 mL/lb/day
Volume:	500 mL or as needed
Frequency:	As needed
Route:	other: SC or IV
Length of treatment/administration:	As needed post-op
Purpose:	Other: Dehydrat on correct on

Drug/Compound Name:	Lidocaine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 - 3 mg/kg

Volume:	1 mL
Frequency:	As needed
Route:	other: iv or sc
Length of treatment/administration:	Post-op day 1
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Metoprolol
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.2 - 2.0 mg/kg
Volume:	Tablet, IV dependent on weight
Frequency:	2x daily
Route:	other: PO or IV
Length of treatment/administration:	As needed post-op
Purpose:	Other: Arrhythmias or heart failure

Drug/Compound Name:	Midazolam
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.3-0.5 mg/kg
Volume:	dependent upon weight
Frequency:	1
Route:	iv
Length of treatment/administration:	duration of surgical procedure
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	N cotine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.1 - 5.0 ug/kg
Volume:	0.5 ml
Frequency:	1-4x
Route:	other: IV or top cal
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Other: Cardiac stressor

Drug/Compound Name:	Phenol
Species:	Dog
Medication Type:	Other
Dose or Concentration:	85%
Volume:	1 ml
Frequency:	1-3 times
Route:	other: intramyocardial - topical
Length of treatment/administration:	1 min
Purpose:	Pre-Operative/Intra-Operative Other: used to interrupt neural fibers of passage

Drug/Compound Name:	Polystyrene m crospheres
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 - 1.0 mL
Volume:	5 mL
Frequency:	Single administration
Route:	other: Intracoronary artery
Length of treatment/administration:	Single administration
Purpose:	Other: Creation of myocardial infarction

Drug/Compound Name:	Post-op fluids (saline/Ringers)
Species:	Dog
Medication Type:	Other
Dose or Concentration:	5 - 60 mL//kg
Volume:	As needed per DLAM DVM guidance
Frequency:	As needed
Route:	
Length of treatment/administration:	As needed post-op
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	potassim chloride
Species:	Dog
Medication Type:	Other
Dose or Concentration:	70-150mg/kg
Volume:	5 ml
Frequency:	once
Route:	iv
Length of treatment/administration:	1-5 min
Purpose:	Other: terminat on

Drug/Compound Name:	Prazosin
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.1 - 0.5 mg/kg
Volume:	1 mL
Frequency:	2-5x
Route:	iv
Length of treatment/administration:	Day of surgery or terminat on
Purpose:	Other: Cardiac stressor

Drug/Compound Name:	Sodium b carbonate
Species:	Dog
Medication Type:	Other
Dose or Concentration:	8.4%
Volume:	10 mL
Frequency:	As needed
Route:	iv
Length of treatment/administration:	Day of surgery or terminat on
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Substance P
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 uM
Volume:	1 mL
Frequency:	1-4x
Route:	other: IV or top cal
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Other: Cardiac stressor

Drug/Compound Name:	Tetramethylrhodamine dextran (TMR-D)
Species:	Dog
Medication Type:	Other
Dose or Concentration:	5-8%
Volume:	100-1000 ul
Frequency:	1 to 20
Route:	other: direct inject on ganglia, nerves, heart
Length of treatment/administration:	30 min to complete inject ons
Purpose:	Other: tract tracing for cardiac nervous system

Drug/Compound Name:	Timolol
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 - 2 mg/kg
Volume:	1 mL
Frequency:	2-5x
Route:	iv
Length of treatment/administration:	Day of surgery or terminat on
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Trazodone
Species:	Dog
Medication Type:	Other
Dose or Concentration:	3-10 mg/kg
Volume:	
Frequency:	2x daily
Route:	oral

Length of treatment/administration:	6 days if needed
Purpose:	Post-Operative

Drug/Compound Name:	Veratridine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1 uM
Volume:	1 mL
Frequency:	1-4x
Route:	other: IV or top cal
Length of treatment/administration:	Duration of terminal procedure
Purpose:	Other: Cardiac stressor

Drug/Compound Name:	Vitamin B12
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1500 mcg
Volume:	
Frequency:	Every 3 days as needed
Route:	sc
Length of treatment/administration:	As needed post-op
Purpose:	Other: Appetite stimulant

Drug/Compound Name:	Wheat germ agglutinin-horseradish peroxidase
Species:	Dog
Medication Type:	Other
Dose or Concentration:	2.5-5%
Volume:	100-1000 ul
Frequency:	1 to 20
Route:	other: direct inject on ganglia, nerves, heart
Length of treatment/administration:	30 min to complete inject ons
Purpose:	Other: tract tracing for cardiac nervous system

Drug/Compound Name:	Yohimbine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 - 2.0 mg/kg
Volume:	1 mL
Frequency:	1-4x
Route:	iv
Length of treatment/administration:	Day of surgery or terminat on
Purpose:	Pre-Operative/Intra-Operative

Euthanasia

For each species used, please provide the euthanasia information. Techniques for euthanasia must follow guidelines established in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

1. **Species:**

   Dog

2. **How will animals be euthanized?**

   Physical Method

3. **For animals that will be euthanized by a physical method, please indicate that method (decapitation or cervical dislocation).**

   a. **Please indicate the appropriate physical method.**

   Other:VF induction (DC current to heart) and/or exsanguination under anesthesia. Heart opened for samples.

   b. **Will anesthesia be used prior to use of the physical method of euthanasia?**

   Yes

   c. **If anesthesia cannot be administered, please provide scientific justification.**

   High dose isoflurane (5%) or alpha choloralose (50 mg/kg) booster just prior to termination.

4. For animals that will not be euthanized at the end of the study, please indicate the final disposition.

All animals undergo terminal procedure to evaluate status of cardiac nervous system and heart

1. Species:

Dog

2. How will animals be euthanized?

Non-Physical Method

3. For animals that will be euthanized by a physical method, please indicate that method (decapitation or cervical dislocation).

a. Please indicate the appropriate physical method.

Other:pentobarbital (100-200mg/kg IV) followed by potassium chloride (70-150mg/kg IV)

b. Will anesthesia be used prior to use of the physical method of euthanasia?

Yes

c. If anesthesia cannot be administered, please provide scientific justification.

NA: Animal will be fully anesthetized and pentobarbital will be added on top of that at termination.

4. For animals that will not be euthanized at the end of the study, please indicate the final disposition.

NA: All animals are euthanized at end of terminal procedures.

Euthanasia Medications

List the drug(s) used for euthanasia on an animal by physical or non-physical methods.

Please note that according to the **AVMA Guidelines for the Euthanasia of Animals: 2013 Edition**, "compressed CO_2 in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable."

Drug Name:	Isoflurane
Species:	Dog
Dose or Concentration:	5%
Route:	inh
Purpose of Drug:	Anesthesia

Drug Name:	pentobarb tal: Veterinary Grade
Species:	Dog
Dose or Concentration:	100mg/kg
Route:	iv
Purpose of Drug:	Euthanasia

Drug Name:	potassim chlor de
Species:	Dog
Dose or Concentration:	70-150mg/kg
Route:	iv
Purpose of Drug:	Euthanasia

Tissue Collection

Please enter the following information regarding tissue collection for the protocol. See ARC Policy on Blood Collection from Laboratory Animals.

1. Tissue To Be Collected:

☑ Blood

☐ Other Collected:

2. Frequency of blood and/or other tissue collections:

Blood samples will be taken pre-op and every three week to following changes in blood biomarkers of cardiac disease. Blood volume for each sample is 2-5 ml. Will be taken via percutaneous venipunture (from jugular or cephalic)

At termination (following euthansia) tissues samples will be taken from the cardiac nervous system, central nervous system, thoracic cavity (including heart and lungs), and viseral organs.

3. Volume of blood and/or other tissue collected per time point:

2-5 ml for blood.

Tissue pieces vary in size at harvest following euthanasia, but are usually about the size of a quarter. This is only at termination.

4. Describe techniques that will be used to collect blood and/or other tissue.

For blood, 2-5 ml from jugular or cephalic vein.

For tissue harvest, tissue dissection with placement of tissue into fixative or flash frozen.

5. Describe how anemia and infection will be prevented.

Blood loss to a 2-5 ml every 3 weeks is not a concern.

Surgical Procedures and Post-Operative Care

Please complete the following questions, noting that any requested exception to ARC Policy must be justified in the space provided.

Note: ARC pol cy requires investigators to employ the following measures to ensure asepsis while conducting survival surgery: asept c surg cal techniques; asept c surg cal field; sterile instruments; clean lab coat/surg cal gown; and sterile surg cal gloves. *For information on surgeries on rodents and birds, please see the **ARC Policy on Survival Surgery in Mice, Rats and Birds**.*

Non-survival surgeries of extended durat on or procedures otherwise likely to increase the risk of Intraoperative infection and/or sepsis (e.g. gastrointestinal surgery) will be evaluated on a case-by-case basis to determine whether aseptic techn ques must be used. Refer to the **ARC Policy on Non-survival Surgical Procedures** for further informat on.

Please note that surgical records are required for all animals. These records must include anesthetic administration and intra-operative mon toring, as well as post-operative recovery observations, including administration of analgesics and antib otics and suture/staple removal if appl cable. Additionally, any adverse outcomes must also be recorded.

1. Pre-Operative care will include (check all that apply):

- ☐ Lab tests
- ☐ Conditioning
- ☑ Fasting: 12 hours
- ☑ Other:

Animals will have a veterinarian assessment before any surgery.

Please note that a phys cal examinat on is required.

2. Will neuromuscular blocking agents be used (e.g., Pancuronium, Succinylcholine)? Refer to the ARC Policy on Neuromuscular Blocking Agents.

No

3. Select all criteria that will be used to assess the proper level of anesthesia.

The level of anesthesia should be assessed on a continuous basis.

- ☑ Respiration rate
- ☑ Heart rate
- ☐ EEG
- ☑ EKG
- ☑ Muscular relaxation
- ☑ Positive toe pinch
- ☑ Corneal reflex
- ☑ Color of mucous membranes
- ☐ Other:

4. Surgical preparation of all mammalian species must include:

1) Removal of hair w th #40 clipper blade in a wide margin around the incis on site.
2) Three alternating scrubs using a germ cidal scrub and 70% alcohol.
3) Placement of lubricating ointment into the eyes.
4) Covering the animal except the surgery site w th a sterile drape.
5) Placing the animal on an external heat source (water circulating heat pad or heating pad set on "low" with a barrier placed between the animal and the heating pad).

- ◉ I assure the ARC that surgical preparation will be performed as outlined above.
- ○ *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

PLEASE NOTE: Any deviation from the policies above must be detailed and scientifically justified in the space below.

5. Indicate the methods to be employed to prevent (a) hypothermia and (b) dehydration (including volume of fluids and route). If this question is not applicable to the proposed surgical procedures, provide a brief explanation.

To prevent hypothermia, the veterinarian recommends the use of water-circulating heading pads over heating lamps and/or electr cal heating pads. The use of heating lamps is strongly discouraged. If not used properly, heating lamps and electrical heating pads may cause thermal injury to the animal. Therefore, describe precaut ons taken to prevent hyperthermia.

Temperature will be monitored via temperature probe during surgery. To prevent hypothermia, circulating water heating pads will be used to keep the dog warm. Additionally, IV hydration using 0.9% normal saline at 3-10 cc/kg/hr will prevent dehydration.

6. Surgical preparation of the surgeon must include:

1) Wash hands w th germicidal soap.
2) Sterile gloves.
3) Surgical Mask.
4) Cap and booties (not required for mice and rats)
5) Sterile gown (clean lab coat or gown acceptable for m ce and rats)

(•) I assure the ARC that surgical preparation will be performed as outlined above.

( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

7. Instrument preparation must be performed by:

1) Autoclave sterilization or ethylene oxide (gas) sterilizat on.
2) Either chemical disinfect on (acceptable between multiple surgeries in mice, rats, and non-mammalian species) or
3) Hot bead sterilizer.

(•) I assure the ARC that instrument preparation will be performed using one of the methods outlined above.

( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

8. Duration of Surgical Procedures (Must be completed as applicable):

For non-survival surgery, ind cate the duration from anesthesia induction to euthanasia. For survival surgery, ind cate the durat on from anesthesia induction to recovery from anesthesia.

Survival: 4-6 hours
Non-Survival: 6-11 hours

9. Provide scientific justification for performing multiple survival surgeries on a single animal.

Multiple survival surgeries will be approved only when they are related components of the experimental design.

There are 2 aspects of this protocol that require multiple surgeries. Aims are defined in experimental design.

For aim 2: Chronic stellate decentralization will be evaluated in the setting of myocardial infarction. This mimics the clinical condition where stellate decentralization is used therapeutically in the setting of established ventricular arrhythmias including ventricular tachycardia storms. The myocardial infarction will be created and 1-3 weeks later the stellates will be removed. The terminal procedure will be performed 1 week to 6 months after MI creation (or stellate decentralization).

For aim 5: The objective of these studies is to determine the structural reorganization of the cardiac nervous system in ischemic heart disease. The protocol involves two survival surgeries: the first involves creation of the myocardial infarct model by coronary artery occlusion; the second survival surgery (4-6 weeks later) involves neural tracers to be delivered into the left or right ventricle (or atria). The terminal procedure will be 1-5 weeks later. Sham MI animals will serve as control for these studies.

10. Please describe all surgical procedures, including non-survival procedures.

The following analgesic regimens will be used for survival procedures:
A. Survival surgeries NOT involving thoracotomy:
1. Pre-operative analgesia: Carprofen (or Meloxicam) and buprenorphine.
2. Sedation and anesthesia: Animals will be sedated with propofol, followed by endotracheal intubation and mechanical ventilation. General anesthesia will be maintained with isoflurane (inhalation therapy). The depth of anesthesia will be assessed throughout surgery by monitoring corneal reflexes, jaw tone, and hemodynamic indices.
3. Post-operative analgesia: Carprofen or Meloxicam every 24 hours for 6 days. Buprenorphine or Buprenorphine SR may be given as needed and in consultation with DLAM veterinarian.
4. Postoperative medications also include
Cefazolin/Cephalexin for 6 days to reduce the risk of infection.
Alternatively, Cefovecin (duration of action 7 days) to reduce risk of infection.
B. Survival surgeries involving thoracotomy:
1. Pre-operative analgesia: Carprofen or Meloxicam, Fentanyl bolus plus IV continuous rate infusion for the duration of the procedure. Marcaine and lidocaine will be injected SQ, IM to induce a regional block. Fentanyl infusion will be maintained in the immediate post-operative phase as feasible.
2. Sedation and anesthesia: Animals will be sedated with propofol, followed by endotracheal intubation and mechanical ventilation. General anesthesia will be maintained with isoflurane. The depth of anesthesia will be assessed throughout surgery by monitoring corneal reflexes, jaw tone, and hemodynamic indices.
Alternatively, animals will be premedicated with benzodiazepine and anesthetized with Etomidate.
3. Post-operative analgesia: Carprofen or Meloxicam every 24 hours for 7 days post-procedure. Buprenorphine SR or Buprenorphine will be given for a minimum to cover the 72 hours post procedure and as needed thereafter. Bup SR has 72 hr coverage. Bup has 6-8 hr coverage.
4. Postoperative medications also include Cefazolin/Cephalexin for 6 days to reduce the risk of infection.
Alternatively, Cefovecin (duration of action 7 days) to reduce risk of infection.
Trazodone may be used as sedative as needed in animals that exhibit signs of hyperactivity in post-operative phase.

Post operative observations to be observed for and reported to the contact PI and veterinary staff include but not restricted to the following:
Palor, increased respiratory rate, decreased appetite, inability to ambulate, not interactive, wound issues (signs of infection including redness, swelling, exudate, warmth), high temperature, dizziness, unsteady gait, impaired vision, hematoma formation/bleeding, nausea/vomiting, pain. The research personnel (including DLAM staff) will be monitoring for any of the above signs, and if witnessed, will inform the PI and the veterinary staff immediately. Nausea/reduced appetite will be treated with anti-emetics (famotidine), pain with analgesics as described above, hematoma with compression, infection with antibiotics, dizziness/unsteady - will be examined for arrhythmias/electrocardiogram checked +/- treated with medications or electrical therapy, increased respiration with diuretic (furosemide), raised temperature with passive cooling with ice and water. If signs are concerning, and cannot be treated with these measures, in conjunction with the veterinary staff, the decision may be made to euthanize the animal to prevent suffering.
The survival surgeries will be performed under aseptic technique and will include preparation of the surgical site with clipping, cleaning with butadiene, and all instruments will be cleaned and sterilized prior to use.

Specific surgical procedures
1. Chronic Closed Chest Myocardial Infarction Induction: (three approaches, only one approach will be used in any given model)
Approach 1: In a canine model we will perform percutaneous coronary occlusion to create a myocardial infarction. Animals will be sedated, anesthetized and monitored as described above. Under general anesthesia, a myocardial infarction will be created by passing a coronary catheter to a main coronary artery. Pre-treatment with anti-arrhythmics such as amiodarone, beta-blockers (esmolol), or lidocaine will be given, depending on anticipated infarct size and arrhythmic potential. Heparin 5000-1000 iu IV will be given prior to insertion of the coronary guide wire and after femoral sheath placement. A coronary guide wire will be passed down the left anterior descending, left circumflex, or right coronary artery and over this an angioplasty balloon and the guide wire removed. Catheter placement will be confirmed by contrast injection. The balloon is inflated to occlude the coronary artery and through the balloon lumen 3-7 mls of polystyrene microspheres (PolybeadR 90µm, Polysciences, Inc., Pennsylvania) are injected. The balloon is deflated and final contrast injection to confirm occlusion of the artery acquired. Then the guide catheter and angioplasty balloon are removed. The animal is monitored for arrhythmias for 20 minutes before removal of the arterial sheath. On removal of the arterial sheath, hemostasis will be achieved by manual compression. Ventricular arrhythmias are expected to occur at the time of myocardial infarction or post infarction. Expected changes in the electrocardiogram include ST elevation or depression as well as T wave inversions as a result of the ischemic event. Sustained ventricular tachycardia or ventricular fibrillation will be treated as per advanced cardiac life support guidelines, ie: external cardioversion (biphasic defibrillation from 150J to 300J), cardiac compressions, and epinephrine IV/intracardiac as required. Brady-arrhythmias rarely occur as a result of LAD occlusion but if they do, then atropine (0.05-3mg/kg IM/IV) will be given as required. Expected mortality related to acute myocardial infarction is approximately 20%.
Approach 2: The animal will be sedated, anesthetized, intubated and ventilated as described above. Using aseptic technique, a left thoracotomy will be performed (T4-T5), a pericardial formed, a coronary artery isolated (right, circumflex or left anterior descending), a suture placed around that artery and the artery occluded in two step procedure: 50% occlusion for 20 min followed by total occlusion. This two-step procedure reduces the potential for ventricular fibrillation. Anti-arrhythmics will be infused prior to vessel occlusion similar to that described above. After observing the animal for ~30 min post occlusion, the chest will be closed in layers, residual air withdrawn using an infant feeding tube temporarily routed from the chest to skin. Following residual air removal, the infant feeding tube is withdrawn with the small incision closed with a purse string suture and the animal recovered as described above.
Approach 3: Same as approach 2 but with the complete coronary artery occlusion only maintained for 90 min. This reperfusion models the clinical condition of stent placement or coronary artery bypass surgery. Anti-arrhythmic therapy, closure techniques and post operative cared is defined above.
An implantable loop recorder for arrhythmia monitoring may be inserted percutaneously (St. Jude Medical, Sylmar, CA) immediately post MI induction. The loop recorder will be implanted on the left sternal edge subcutaneously. The area will be prepped with betadine wash and draped to ensure sterility of the area. The implant area is pre-medicated with local anesthesia (lidocaine 2%). A small incision of 2-3cm will be made into the subcutaneous region and a pocket for placement of the device created. Following device placement, the tissue will be closed in layers. Post-operative care is defined above.
The terminal procedures on these animals will be 1 week to 6 months after MI creation.

2. Chronic stellate decentralization: This will be performed in canines at approximately 1-3 weeks post myocardial infarction or in normal canine models as a treatment control. See section 1 for methods to produce chronic myocardial infarction. The following methodology is to chronically decentralize stellate ganglia from the spinal cord. In a canine model, animals will be sedated, anesthetized and monitored as described above. A lateral thoracotomy or video assisted thoracotomy (VATS) procedure as described below will be done for stellectomy.
a) Lateral thoracotomy: This will be performed in the 3rd or 4th intercostal space. An incision will be made in the subcutaneous tissue, and then the muscle and nerves carefully isolated to avoid disruption, till we reach the ribs. The intercostal muscles will be isolated via an incision in the superior aspect of the rib and retractors inserted to open the thorax. A 4-5cm incision will be made in the left parietal pleura and the cardiac sympathetic chain isolated. The lower third of the stellate to T4 paravertebral ganglia will be removed surgically. Alternatively, the lower third of the stellate to T2 paravertebral chain will be removed. The left lung will be re-inflated. The incisions will be sutured closed in layers. In some animals, the same procedure will then be repeated on the right side. For other animals only unilateral stellectomy will be performed. In either case, residual air will be withdrawn from the chest cavity using an infant feeding tube temporarily routed from the thoracic cavity to the skin surface. Following removal of that residual air the infant feeding tube will be removed and the small incision closed with a purse string suture. Following the procedure the animals will be closely observed for potential complications such as wound healing, pain, and pneumothorax. Post-operative analgesia will be administered as described above. If signs of pain are apparent this will be addressed in consultation with the veterinary staff and may include intercostal nerve block.
b) VATS: Three 1.5cm incisions will be made in the intercostal spaces of the chest on the right and left side. Each side will be removed separately. For left cardiac stellectomy the contralateral lung will be intubated and ventilated and the ipsilateral lung deflated. The VATS instruments will be introduced via the small intercostal incisions. A 4-5cm incision will be made in the left parietal pleura and the cardiac sympathetic chain isolated. The lower third of the stellate to T4 paravertebral ganglia will be removed surgically. Alternatively, the lower third of the stellate to T2 paravertebral chain will be removed. The left lung will be re-inflated and the VATS instruments removed. The incisions will be sutured closed in layers with absorbable sutures and residual air withdrawn with an infant feeding tube temporarily inserted into the thoracic cavity during closure. The same procedure may then be repeated on the right side. Following residual air withdrawal, the infant feeding tubes will be removed with the residual opening closed with a purse string. In some animals only unilateral stellectomy (right or left) will be performed; others will have bilateral stellectomy. Pre-operative and post-operative

analgesia will be administered as described above. Following the procedure the animals will be closely observed for potential complications such as wound healing, pain, hemothorax, and pneumothorax. If signs of pain are apparent this will be addressed in consultation with the veterinary staff.
The terminal procedure will be 1 week to 6 months after MI creation (or stellate decentralization).

3. Chronic bioelectronic implants
This will involve different bioelectronic implants. The preparation, intubation and anesthesia are described above. Animals will receive one of the three therapeutic options (Vagal nerve stimulation [VNS]; spinal cord stimulation [SCS] or axonal modulation therapy (AMT) using intrathoracic multi-pole electrodes and associated implantable programmable stimulators /generators (IPG's). Pacemakers and implantable cardiac defibrillators (ICD) may be implanted based upon therapeutic criteria for management of MI-induced arrhythmias. Interfaces for monitoring cardiac or neural activity may also be implanted. Terminal procedures will be 1 week to 6 months after MI creation and/or bioelectronics implant. Bioelectronic implants will be done in conjunction with MI induction or by themselves if the animal is a sham control.
a) Pacemaker or implantable cardiac defibrillator implantation: A 4-5cm incision will be made one inch subclavicular after soaking with betadine, draping to ensure sterility, and insertion of local lidocaine 2% subcutaneously. An 18 guage percutaneous needle will be inserted into the extrathoracic subclavian vein and a pacemaker lead insertion is made through this to the atrium and/or ventricle. A passive or active fixation lead will be then connected to the pacemaker generator (or ICD) and tested to ensure threshold, capture and output are adequate. The pocket will then be closed in layers. Pre-operative and post-operative analgesics will be administered as described above.
b) Vagal nerve electrode and IPG: Local lidocaine 2% will be injected subcutaneously into the sterilized (betadine) and draped ventral neck area. An incision of 4-5cm will be made in the left (and/or right) ventral neck region and the cervical vagosympathetic nerve trunk isolated via a lateral neck cut down. The vagosympathetic trunk will be separated from the common carotid artery and sterile multi-polar electrodes will be placed around the cervical vagal trunk. The lead is then tunneled subcutaneously to IPG(s) placed in a small pocket(s) created subcutaneously in the subclavicular space or dorsal aspect of the neck. The incisions will be closed in layers. Pre-operative and post-operative analgesics and antibiotic therapy will be administered as described above. Wound healing will be monitored as described above.
c) Spinal cord electrode and IPG: The dorsum of the canine will be prepped with betadine and draped to ensure sterility. Following local administration of lidocaine 2% (T4-T8 region) a small incision will be make over the spinal processes. An epidural needle,18 Gauge, will be inserted into the epidural region using a loss of resistance technique. Once the epidural region is accessed the spinal cord electrode will be inserted via the central lumen of the epidural needle and advanced to the C8-T4 spinal level. Confirmation of position of the electrode will be via fluoroscopy. The lead will be tunneled to a subcutaneous generator in a pocket fashioned from a 3-4 cm incision located on the dorsal surface of the lower thoracic or upper lumbar region. The SCS electrode and IPG will then be tested to ensure functioning and the tissue closed in layers. Pre-operative and post-operative analgesics and antibiotic therapy will be administered as described above. The wound healing will be monitored as describe above.
d) Paravertebral/stellate electrode and IPG: A lateral thoracotomy will be performed in the 3rd or 4th intercostal space. An incision will be made in the subcutaneous tissue, and then the muscle and nerves carefully isolated to avoid disruption, till we reach the ribs. The intercostal muscles will be isolated via an incision in the superior aspect of the rib and retractors inserted to open the thorax. The cardiac sympathetic chain (T1-T5) will be isolated. The electrode will be wrapped around the T1-T2 paravertebral chain (or stellate ganglia) and the lead tunneled to a pocket fashioned from a 3-4cm incision in the subcutaneous fascia. Alternatively this electrode can be placed around the ansae subclavia, middle cervical ganglia, or vagosympathetic nerve trunk. A second electrode may be placed on the T2-T3 region to stimulate sympathetic nerves or cervical vagus to stimulation parasympathetic nerves. The lead(s) will be tunneled from the thoracic cavity connected to a skin button place between the scapula. The skin button function as the interface connector between the electrodes and external instrumentation. Alternatively, the leads will be connected to a dual channel IPG that is placed in a subcutaneous pocket created on the dorsal aspect of the animal. A chest tube will be temporarily placed and routed to the skin surface. The lead and IPG will then be tested to ensure functioning and the tissue closed in layers (chest, IPG, skin buttons). At the end of the surgical procedures, residual air will be withdrawn using the chest tube; that tube is then removed with the incision closed with a purse-string suture. The cutaneous skin buttons will be protected by stockingette and/or fishnet protective vests. The wound healing will be monitored closely. Skin buttons, if used, will be checked daily, cleaned and treated if signs of irritation are noted. Pre-operative and post-operative analgesics and antibiotics will be administered as described above. If signs of pain observed, this will be addressed in consultation with the veterinary staff. Alternatively, the electrodes can be deployed using the VATS approach as defined above (see section 3b).
e. Bioelectronic monitoring interfaces. The animal will be sedated, anesthetized, intubated and ventilated as described above. Using aseptic technique, a left (or right) thoracotomy will be performed (T3-T4, T4-T5 or T5-T6), a pericardial formed. A sterile thin-film multichannel array will be placed on the atria and/or ventricle surface, the pericardium closed and the lead tunneled out through the chest wall to a skin button placed on the dorsal region of the animal (mid scapular level). This button serves as the interface between implanted interfaces and extrinsic control devices. A thin-film 2D plunge electrode may also be inserted into the stellate ganglia and that lead tunneled out through the chest wall to a skin button place on the dorsal region of the animal (mid scapular level). Alternatively, the cardiac and neural interfaces will be connected to an implantable device capable of providing telemetry of bioelectric signals. This device will be placed in a subcutaneous pocket in the mid-scapular region. The chest will be closed in layers, residual air withdrawn via a temporarily implanted chest and the animal recovered as described above. The cutaneous skin buttons will be protected by stockingette and/or fishnet protective vests. Skin buttons will be checked daily, cleaned and treated if signs of irritation are noted.

4. To determine the structural organization of the cardiac nervous system, neural tracers will be injected into the heart, peripheral ganglia or nerves of the cardiac nervous system. Tissues will be harvested one to five weeks later (depending on tracer). In a subset of animals, they will first undergo myocardial infarction and 2-6 weeks later neural tracer injections will be performed. Time matched controls will likewise be done. Tracer injections will be done using either an intravascular route or following surgical exposure of the injection site.
For intravascular delivery of tracers, using aseptic techniques, a sheath will be placed percutaneously into the femoral artery and neural tracers will be delivered via a Myostar catheter in the left or right atria or ventricles. On removal of the arterial sheath the artery will be managed by manual compression for at least 10 minutes. Post-operative care is as specified above.
Alternatively, the animal will be anesthesized, intubated, ventilated and a unilateral thoracotomy (T3-T4, T4-T5 or T5-T6) done. A pericardial cradle will be created and tracers micro-injected into multiple sites of the cardiac muscle using Hamilton Syringes. Following completion of tracer injections, the pericardium will closed and the chest sutured closed in layers. Residual air withdrawn from the thoracic cavity via a chest-tube temporarily placed on closure. The chest tube will be removed following aspiration of the residual air. Post-operative

analgesia and antibiotic therapy will be administered as described above. If signs of pain are apparent this will be addressed in consultation with the veterinary staff. The terminal procedure will take place 1-5 weeks later (depending on tracer) and will involve harvest of tissues (see section L, terminal procedures).
Tracer injections will be made into multiple sites of peripheral autonomic ganglia, peripheral nerves or directly into organs (heart, lungs, etc). Each injection will involve up to 100-1000 µl of volume. Tracers to be utilized will be selected from the following list:
CTB: Cholera Toxin subunit B conjugated with a fluorophore (CTB-Axexa Fluor)
Wheat germ agglutinin-horseradish peroxidase (WGA-HRP)
Cholera toxin subunit B- horseradish peroxidase (CTB-HRP)
Fluoro-Gold
1,10-dioctadecyl-3,3,30,30 tetramethylindocarbocyanine methanesulfonate (DiI)
Tetramethylrhodamine dextran (TMR-D)

Termination procedures for canine models
A) Procedural intubation, anesthesia and preparation: The animals will be pre-medicated, sedated and anesthetized as described above. After endotracheal placement the animals will be ventilated and anesthesia maintained with isofluraneduring the surgical preparation. Oxygen saturations will be monitored, as will heart rate and end-tidal C02. A temperature probe will be placed in the rectum and water-circulating heating pads used to assist in temperature control. During active surgical procedures, intermittent boluses of fentanyl will be administered prior to thoracotomy. In studies where laminectomy is performed a continuous infusion of Fentanyl will be given for the duration of the surgical preparation. After completion of the surgery the anesthesia will be transitioned to α-chloralose. This is necessary as isoflurane can blunt cardiac reflexes (see below for further justification). Alpha-chloralose will be filtered through a 0.22um filter prior to use. The depth of anesthesia will be monitored constantly for withdrawal reflex, eye-blink, jaw tone, abdominal muscle tone and in conjunction with heart rate, blood pressure, temperature and cardiac electrical function measurements. Adjustments will be made to anesthesia as necessary to maintain a surgical plane of anesthesia. Femoral artery (or arteries) will be cannulated either directly or with a sheath to allow for monitoring systemic blood pressure or for insertion of electrophysiological (or hemodynamic) catheters. The femoral vein (or veins) will be cannulated to administer fluids and medications or with a sheath to allow for placement of electrophysiological (or hemodynamic) catheters. The carotid artery will be cannulated with a sheath to allow for insertion of the hemodynamic (or electrophysiological) measuring catheter. End-tidal CO2 will be maintained between 35-40 mmHg. Arterial blood gas sampling will be performed to ensure maintenance of adequate oxygenation. Adjustments to tidal volume along with bolus administrations of appropriate doses of sodium bicarbonate will be given as required to maintain acid-base status.
B) Surgical Preparation (chest and neck): The surgical preparation involves a transthoracic sternotomy (T4-T5 or T5-T6) to expose the heart, the stellate ganglia, paravertebral sympathetic chains and other intrathoracic elements of the cardiac nervous system including the intrinsic cardiac nervous system. In the neck, the cervical vagus will be isolated bilaterally if required. After surgical preparation there will be a stabilization period of 30 minutes. (see section L for termination procedures and end of experiment)
C) Hemodynamic indices: To measure left ventricular pressure a Millar catheter (Millar Instruments, Houston, TX) will be inserted via the left ventricular chamber from the carotid (or femoral) artery.
D) Electrophysiological Assessment: A 12-lead posterior electrocardiogram will be recorded via the Prucka CardioLab system (GE Healthcare, Fairfield, CT). A custom made 56-electrode sock, 64 electrode plaques, or multipolar catheters, will be placed over both ventricles to measure unipolar epicardial electrograms derived from the ventricular epicardium. Alternatively, thin-film micro-arrays will be placed on (or into) the atrial and/or ventricular surfaces. Alternatively, a basket multi-electrode catheter may be placed for endocardial mapping. Electrophysiology protocols to assess atrial and ventricular conduction patterns, propagation, activation sequence and inducibility of arrhythmias may be performed using programmed cardiac pacing from the atria and/or ventricles.
E) Cardioneural mapping: High density grid electrodes (epicardial or endocardial) will be used for regional cardiac electrophysiological assessment. In association with cardiac electrophysiological recording, flexible penetrating microelectrode arrays will be inserted into one or more of the autonomic or primary sensory ganglia and/or directly into atrial and/or ventricular muscle. Neural and cardiac activity will be acquired with high density data acquisition systems (e.g. Neuronexus Smartbox, Blackrock or Cambridge Electronic Design). The cardiac autonomic ganglia included in the neural investigation will include intrinsic cardiac, stellate, middle cervical, mediastinal, nodose, and dorsal root ganglia. In addition, following a laminectomy (see section F4), neural activity will be recorded from surface and from penetrating arrays place over and inserted into the spinal cord from the dorsal surface.
F) Neural Stimulation
1. Sympathetic Ganglia Stimulation: Stimulating electrodes will be inserted into (or around) the paravertebral, stellate, middle cervical or mediastinal ganglia. Stimulation of the sympathetic ganglia will be with an electrical stimulator at frequencies ranging from 1-20Hz, pulse widths of 1-4ms, and intensities from 0.2-15mA.
2. Bioelectronic blocking of autonomic nerves. Multiple-pole electrodes will be placed in or around autonomic nerve projections in the neck (e.g. vagosympathetic trunk) and chest (e.g. paravertebral ganglia, ansae subclavia, vagosympathetic trunk). Focal nerve blocking will be accomplished with high frequency alternating current, DC current, or hybrid electrodes that utilize both AC and DC current. As an alternative, thermal (cryo) catheters will be placed on these same locations to produce focal decreases in temperatures. Stimulating electrodes will be placed rostral and caudal to the blocking site to assess the impact on afferent and efferent control of cardiac function. Efferent blockade will be assessed using graded stimulation of sympathetic (including paravertebral) ganglia and assessing changes in cardiac electrical and mechanical function. Afferent blockade will be assessed by direct neural recordings from peripheral autonomic ganglia or the spinal cord.
3. Cervical vagus stimulation: Multi-polar cuff electrode will be wrapped around each cervical vagus or by bipolar electrodes inserted into the vagus trunk. Cervical vagus stimulation will be assessed by using an electrical stimulator at frequencies from 1-20Hz, pulse widths of 0.2-5ms, and intensities from 0.25-15mA. Cardiac electrophysiological changes and autonomic neural activated will be recorded.
4. Spinal cord stimulation: This can be performed via epidural closed access or open laminectomy. Briefly the epidural insertion of spinal cord stimulation electrodes will be inserted around T5-T8 region after local administration of lidocaine 2%. An epidural needle, 18Guage, will be inserted via a loss of resistance technique. Once the epidural region is accessed the spinal cord electrode will be inserted via the central lumen of the epidural needle. Confirmation of position of the electrode will be via fluoroscopy. The usual electrode position is between C8 to T5. The alternative open access procedure requires a laminectomy. We will perform a laminectomy from C8 to T4. The skin will be injected with lidocaine for local anesthetic. Fentanyl (20-30mcg/kg IV bolus) will be administered prior to opening the skin and will be constantly infused (20-30mcg/kg/hr IV) throughout the surgical procedure (before transitioning to alpha-chloralose when all surgical procedures are completed). After careful dissection of the tissue, the spinous process will be removed to access the spinal cord. The spinal cord electrode is then placed directly on the spinal cord. This electrode is either multi-pole, paddle or thin-film 2D array type. SCS stimulation protocols will include tonic (1-999 Hz), burst and/or high frequency (1000 to 10000 Hz) usually delivered at sub- or at motor threshold intensities. Motor threshold is defined as the lowest current sufficient to produce contraction of muscle groups in the sphere of influence for that segment of the cord.

G) Chemical stimulation of neurites: Chemicals will be infused into the atria, coronary blood vessels, intravenously, or epicardially to activate the cardiac nervous system. Increases or decreases in blood pressure of 20mmHg are expected at the higher end of the dose range for several of these agents. See Experimental Design section for a list of specific agents, and the Medications section for doses and routes of administration.
H) Cardiac autonomic stressors: These will involve transient physical touch of various regions of the heart and lungs along with transient occlusion of the inferior vena cava, descending aorta, and/or various of the coronary arteries. Such interventions will be done directly and/or percutaneously. Baseline and evoked changes in cardioneural function will be evaluated. Cardiac stress will also be induced using program pacing from multiple points on the cardiac surface or within cardiac muscle.
I) Neurochemical markers: Microdialysis catheters will be inserted into the myocardium to obtain periodic samples of interstitial fluid. Catheters will be perfused and samples collected. Alternatively, small diameter wires (50 to 150 micrometers) or small diameter plunge electrodes with multiple sensory will be placed directly into the myocardium and the cardiac microenvironment assessed by fast scanning (low current) voltammetry or with capacitative immuno-probes. Alternatively, the chemical sensors will be place intravascular.
J) Ablation of ganglia and myocardium: Ablation of electrical circuits within the myocardium or intrathoracic ganglia will be performed to define the functional control of regional cardiac function. Efficacy of ablation will be assessed based on changes in basal and evoked response to stimulation of extracardiac sympathetic and parasympathetic nerves. Ablation may be accomplished by surgical, chemical (phenol), Radio-frequency (RF) or cryo-ablation.
K) Termination: the animal will be sacrificed using pentobarbital followed by potassium chloride or alternatively VF induction with low voltage (~9 volts) direct current under inhaled isoflurane.
L) Tissue collection: For cardiac MRI imaging studies: gadolinium will be administered 20 minutes pre-termination. For histological, immuno-histochemical and molecular studies of the cardiac nervous system and heart the following tissues may be explanted: heart (including intrinsic cardiac ganglia), mediastinal ganglia, stellate ganglia, vagus nerves, paravertebral chain, the middle cervical ganglia, the nodose ganglia, the spinal cord and the dorsal root ganglia. Nervous tissue may be harvested just prior to euthanasia and under surgical plane anesthesia. Following euthanasia, additional samples make by taken from the central nervous system or viscera to assess multi-organ impact of cardiovascular disease.

Rationale for alpha-chloralose use:
Alpha chloralose is the preferred anesthetic of choice for acute studies for evaluation of autonomic function in normal and controlled cardiac pathology states. It maintains a high level of basal sympathetic and parasympathetic tone that can be reflexly modified by afferent inputs and responds in robust ways to direct activation of autonomic inputs. It is our experience (1-3, 5, 6) and that of others (multiple published papers) that alpha chloralose can provide an adequate level of surgical anesthesia and that it is superior to isoflurane in maintaining basal autonomic tone, especially parasympathetic. This was recently verified the following reference:
[redacted]
The autonomic nervous system itself is a good barometer of the homeostatic state. If pain afferents are activated there is sympatho-excitation leading to tachycardia and hypertension (4). In our various experimental interventions (touching the heart, applying neurochemicals to sensory fields, bioelectric stimulation, etc) such responses are not evoked. All objective criteria: jaw tone, eye-blink, withdrawal reflex to toe pinch, etc are likewise maintained stable. Blood pressures and heart rate are maintained in physiological range for multiple hours and autonomic neural responses function normally. No muscle relaxants are used in our experiments to avoid masking effects of inadequate anesthesia. With our anesthetic technique that has been perfected over several years, we do not observe any withdrawal or movement response to a surgical/painful stimulus, further attesting to the adequacy of the plane of surgical anesthesia. It is also worth mentioning that alpha chloralose is not an immobilizing agent.
One of the main themes in this protocol is to study the impact of the autonomic nervous system on cardiac electrophysiology in normal and pathological conditions. An intact and functioning autonomic nervous system with active reflexes is required. Alternatives to alpha-chloralose (e.g., ketamine, fentanyl, midazolam) are well recognized to dampen autonomic nervous system function in a manner that interferes with functional autonomic reflexes (see references below). Alpha-chloralose, with a long history in autonomic neuroscience, is known to maintain autonomic function while maintaining an adequate level of anesthesia. For the specific purpose of having the cardiac autonomic nervous system as intact as possible, no satisfactory alternatives to alpha-chloralose exist.

With regards to the suppressive action of opiates such as morphine on autonomic neurons, the references below directly address this issue.
[redacted]

With regards to the suppressive effect of ketamine on autonomic nervous system function, the references below address this point.
[redacted]

With regards to the effect of midazolam/benzodiazepines, the references below address this point.
[redacted]

With regards to our documented experience with alpha chloralose, the following are representative publications.
[redacted]

[redacted]

Potential side effects of medications used:
1. Potential side effects of anesthetics:
Hypotension, sedation, respiratory depression, dizziness, redness, vomiting, bradycardia/tachycardia, agitation, muscle tremors, seizures, hypertonicity
2. Potential side effects of analgesics:
Dizziness, sedation, vomiting, gastrointestinal upset, redness, loss of appetite, abdominal pain secondary to constipation, respiratory depression
3. Potential side effects of anti-arrhythmics:
Arrhythmias, tachycardia/bradycardia/ventricular arrhythmias, hypersensitivity, atrioventricular conduction block, dizziness, reduced appetite, skin discoloration (amiodarone), agitation/seizures (lidocaine toxicity), hypotension.
4. Potential side effects of beta-agonists:
Bradycardia, hypotension, dizziness, altered appetite, heart failure
5. Potential side effects of beta-sympathomimetics:
Hypertension, hypotension, tachycardia, atrial/ventricular arrhythmias.
6. Potential side effects of anti-coagulants:
7. Bleeding. Protamine may be used to reverse the effects of heparin 1-1.5mg/100units heparin given.

11. Please indicate the suture materials to be used:

- ☑ Internal: absorbable sutures (e.g., Dexon, Vicryl)
- ☑ External: non-absorbable skin sutures (e.g., Nylon, wound clips). *Please note that external skin sutures or wound clips must be removed 7-14 days following surgery.*
- ☑ Other/not applicable (describe below):

NA for terminal studies.

12. During recovery from anesthesia, what indications will be monitored to assure the animals are stable?

In accordance with the Guide for the Care and Use of Laboratory Animals, particular attent on should be given to thermo-regulation, card ovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.

See question 10 for complete description of pre-operative, intra-operative and post-operative care and monitoring.

13. How often will animals be monitored after anesthetic recovery?

The ARC requires that animals be observed continuously by trained personnel during the immediate anesthet c-recovery period (i.e., until the animal is ambulatory) and at least daily after anesthetic recovery. However, post-operative mon toring frequency may be greater depending on the complex ty of procedures involved, administration of post-operative analgesia, and the species of animal used.

See question 10 for complete description of pre-operative, intra-operative and post-operative care and monitoring.

We maintain continuous monitoring of heart rate, blood pressure and pulse Oximetry during surgical procedures, hourly for the 6-9 hours after surgery and longer if the condition of the animal dictates. After this time, and within the initial 24 hour post-op recovery period, between monitoring times are increased. For the initial surgeries, one of the authorized personnel will observe the animal status ~6 hours from the end of the hourly evaluations; this is usually during the overnight hours and within the therapeutic window for analgesia. In consultation with DLAM Vets - if adequate analgesia and stability is verified at this ~6 hour time point, that time window (in the first 24 hour post-op period) will be moved to ~10 hrs. During the 1st week post-operative period, animals are monitored by one of the authorized personnel 2x daily (and more often if the condition of the animal dictates). Thereafter, animals are monitored daily by one of the authorized personnel as listed in the protocol. If blood gas measurements are required we have an iSTAT handheld blood analyzer.

Species Surgery

Species:	Dog
Number of Animals:	142
Surgery Type:	Survival Surgery
Surgeries per Animal:	
Time Between Surgeries:	

Species:	Dog
Number of Animals:	36
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	2
Time Between Surgeries:	3-6 weeks

Species:	Dog
Number of Animals:	258
Surgery Type:	Nonsurvival Surgery
Surgeries per Animal:	
Time Between Surgeries:	all terminated at end, 80 are acutes

Non-Surgical Procedures

1. Describe the basic methods used for all non-surgical manipulations (e.g., imaging, behavioral studies, Parkinson's and diabetes induction, chronic implant maintenance, cannulation).

Echocardiogram. Long and short-axis electrocardiograms will be obtained from unanesthetized animals as they are lying on their right or left sides. Animals will be gently restrained by hand as the echocardiograms are obtained. Echocardiograms are obtained at baseline and periodically during the progression of cardiac disease. Animals will be imaged from the right and left sides to obtain short-axis and long-axis (4 chamber views of the heart.

Pavlov stand. Objective is to obtain baseline electrocardiograms in unanaesthetized and unstressed (quiet) states during the progression of cardiac disease. In animals with chronic implant of the cardio-neural mapping system, this time will also be used to assess activity as recorded from the implanted electrodes. In animals with bioelectronic implants, devices will be assessed and re-programmed while the animal are standing quietly in the Pavlov stand. Prior to initiation of recordings, animals will be acclimatized to the pavlov stand, starting with short exposures with food reward and increasing time of exposure to the required maximum of 2 hours in the stand. No food restrictions are required.

Holter recordings: 24 hour holter monitoring will be done periodically (~1-2 times/month) from our animal models at baseline and in response to biolectronic therapies. Holter monitors are affixed to jackets. Stockingnet and or dog jackets (Chapman) may be placed over holter jacket to help protect against animal chewing on attached wires or Holter recording device.

2. List probable clinical responses to and potential complications of the nonsurgical procedure(s).

None expected.

Gas Anesthetic

NOTE: Gas anesthetics like isoflurane, halothane, enflurane, and ethane must be used safely. The Off ce of Environment, Health & Safety (EH&S) requires the use of a certified fume hood or a gas anesthet c machine that contains a scavenging dev ce (e.g., anesthet c gas machine w th charcoal filter; ducted fumehood or ducted b osafety cabinet; Crump WAG System; vaporizer w th a scavenging filter, such as F-air canister) when using gas anesthetics.

1. What gas anesthetic agent(s) will be used?

- ☐ Halothane
- ☑ Isoflurane
- ☐ Other:

2. Gas anesthetic(s) will be scavenged via:

- ☐ Certified Fume Hood:
- ☑ Other: canister scavenging on outflow line

Scavenging Location

Building	Room
[redacted]	[redacted]

Hazardous Agents

If you are planning to use rDNA, chemical or biohazardous agents (carcinogenic, teratogenic, or highly toxic substances; nanoparticles; human cell lines; or infectious agents) in live animals, you are required to provide the information about the agents below. The appropriate safety committee will review your request directly in the application.

Agent(s) that will be used:

Agent Name	Route of Administration	Volume	Time to Euthanasia	Approval Date
atenolol	iv	1-10 ml	6 hours	3/5/2015
atropine	iv	1-10 ml	6 hours	3/5/2015
Enalapril	oral	tablet 0.25-1.00mg/kg	2 to 4 weeks	3/4/2015
fentanyl	iv	2-10 mcg/kg/hr for up to 4 hr	NA	3/4/2015
Nicotine	Intra-coronary or cardiac surface	1 ml	6 hours	3/5/2015
para-formalydehyde	tissue preservation	50 ml	after euthanized	3/5/2015
Prazosin	iv	1 ml	6 hours	3/4/2015
Veratridine	top cal on heart	2 ml	6 hours	11/8/2017
yohimbine	iv	1-10 ml	6 hours	3/5/2015

Prolonged Physical Restraint

See ARC Policy on Physical Restraint of Unanesthetized Animals. ARC policy defines prolonged physical restraint as restraint for longer than 15 minutes. It is NOT necessary to complete this section when the physical restraint is: (1) for brief restraint/examination, (e.g., for collection of samples or for injections), or (2) for an anesthetized animal. If devices such as restraint socks or squeeze cages are used, it is important that such devices be suitable in size and design for the animal being held. They must operate properly to minimize stress and avoid injury to the animal.

1. Rationale for Restraint:

Echocardiography and moderate duration evaluation of heart rate control with and without bioelectronic nerve stimulation requires animals be evaluated with moderate restraint. Pavlov stand is a sling with 4 holes, one for each leg. Often animals fall asleep during 1-2 hour recording sessions.

2. Describe the type of restraint device, dimensions, conditioning of the animal to restraint, etc.

Echocardiogram. Long and short-axis electrocardiograms will be obtained from unanesthetized animals as they are lying on their right or left sides. Prior to initiation of echo recordings, animals will be acclimatized to the lying on either side with gentle restraint of their upper and lower extremities. Echocardiograms are done in a quiet low-light room with three persons, two on restraint and one on echo. Echocardiograms are done on a echo table with holes that allow access points to the chest wall. Maximum time for echo does not exceed 10 min. Light scratching behind ears and gentle words/praise are usual positive reinforcement. At all times when a canine is in the echo room, there is/are authorized personnel in the room with that animal. Echocardiograms are obtained at baseline and periodically during the progression of cardiac disease.

Pavlov stand. Objective is to obtain on baseline electrocardiograms in unanaesthetized and unstressed (quiet) states during the progression of cardiac disease. Prior to initiation of recordings, animals will be acclimatized to the restraint system of choice (the Pavlov sling/stand), starting with short exposures (30 min) with food reward and increasing time of exposure (in 30 min increments) to the required 2 hour. At all times when a canine is in the stand/sling, there is authorized personnel in the room with that animal. In many instances, the animals even go to sleep while in the stand/sling.

3. Restraint Duration and Frequency:

NOTE: The period of restraint should be the minimum required to accomplish the research objectives.

1-2 times/week.
10 min for echo
1-2 hours for heart rate evaluations
Holter: 24 hour, but free-ranging.

4. Describe how frequently the animals will be observed during the restraint period.

Please also describe criteria for removal of animals from restraint.

For Echo and recordings in Pavlov stand, animals are under constant observation.
"Animals "fail" the Pavlov Stand training if they remain fidgety during training session and are unable to remain "calm". An animal that is trained to the Pavlov stand will usually exhibit a marked sinus arrhythmia during standing and often time are so relaxed that they tend to dose off in the Pavlov stand. If the animal proves unsatisfactory for such conscious baseline recordings, they will be shifted to either chronic protocols that do not require the Pavlov stand (e.g. tracer injections for tract tracing) or to acute procedures."

5. Will pain or discomfort be induced?

Yes

At higher level bioelectronic nerve stimulation animals can potentially experience some sensation. If discomfort is noted (e.g. animal become agitated during on phase especially in association with concurrent tachycardiac), stimulus intensity will be immediately reduced or stimulation stopped.
No discomfort with Echo, Holter or basal conditions in Pavlov stand.

Species Restraint

Species	Number of Animals
Dog	170

Principal Investigator Assurance

After you have reviewed and answered yes to the items below, please click "Save" at the bottom of the page. Please note that the PI must complete this section. To determine your eligibility to serve as Principal Investigator of a research protocol, please refer to UCLA Policy 900 (Principal Investigator Eligibility) or contact the ARC administrative office (310-206-6308). If the terms of Policy 900 are not met, faculty sponsorship or principal investigatorship by a UCLA employee with faculty appointment may be required.

Regarding policies governing animal research at UCLA:

Yes	No	
◉	○	I agree to abide by all applicable federal, state, and local laws and regulat ons and UCLA pol cies and procedures.
◉	○	I am aware that deviat ons from an approved protocol or violations of applicable pol cies, gu delines, or laws could result in immediate suspension of the protocol.
◉	○	I understand that the attending veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.

◉	○	I declare that all experiments involving live animals will be performed under my supervis on or that of another qualified scientist. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.
◉	○	I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment or illness.
◉	○	I declare that the information provided in this appl cation is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this appl cation accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
◉	○	Any modificat ons to the protocol will be subm tted to and approved by the ARC prior to initiation of such changes.
◉	○	The experimental design has been refined in order to minimize the invasiveness of the proposed procedures.
◉	○	I assure that the proposed research does not unnecessarily duplicate previous experiments.

Agreement on electronic submission:

I understand that by submitting this document that this document will be sent to appropriate members for review. I further understand that once submitted for review, this protocol cannot be modified or changed unless so requested by the ARC. In addition, once approved, all changes or modifications must be subm tted for review and approval of the ARC prior to in tiation.

Completed by: [redacted] **8/2/2019**

FS Assurance

This section is empty.