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:	2/4/2019-12/19/2019
Received Date:	12/3/2018
Type:	Amendment
Species:	10 Dog (Pain Category D)
Create Date:	11/17/2018 3:31:21 PM
Created By:	[redacted]
Owner:	[redacted]

Updated Sections
Amendment Summary
Personnel
PI Assurance
Pre-Review

Personnel Certifications Due:

[redacted]
- General Certif cation Test (expired on 11/2/2019)
- Species Specific Training for Dog

[redacted]
- General Certif cation Test (valid until 12/9/2019)
- Species Specific Training for Dog

[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 Dr [redacted] and Dr. [redacted] will receive species-specific training by Dr. [redacted] or [redacted] designee so that training may be tailored to the animal model being studied. Dr. [redacted] will inform the ARC when this training has been completed.

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.**

We are adding two additional co-investigators to assist with data analysis and other facets of the research described in this protocol.

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:	[redacted]	**Dept:**	[redacted]
Status:	Faculty		

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.

Has performed diagnostic and interventional neuro-angiography on animals including dog at [redacted] 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.

1. Femoral artery access for diagnostic cerebral angiogram under X ray
2. Intracerebral injection of autologous blood by image guided percutaneous access
3. Femoral artery access for interventional cerebral angiogram under X ray, use of endovascular sympathectomy device in basilar artery

Will this person handle radioactive materials or radioactive animals?

No

Co-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?

Co-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?

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.

Worked with mice including intracerebral survival surgery and small animal MRI at [redacted] since [redacted]

Will initially learn specific canine survival surgery techniques by observing PI.

Has performed neurosurgical procedures in humans as a Neurosurgery resident at [redacted] from [redacted]

Has performed diagnostic and interventional cerebral angiography in humans at [redacted] as a resident and fellow of [redacted] since [redacted]

Has not worked with dogs previously. [redacted] will be trained/monitored by PI and DLAM staff.

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

1. Femoral artery access and diagnostic cerebral angiogram under X ray
2. Intracerebral injection of autologous blood by image guided percutaneous access
3. Femoral artery access for interventional cerebral angiogram under X ray, use of endovascular sympathectomy device in basilar artery

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-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?

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.

[redacted] is a neurosurgeon who practices in [redacted] currently serving as a research fellow in the division of [redacted] at [redacted] has extensive experience working with animal models in the research setting.

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

Data collection and analysis, including statistics.

Will this person handle radioactive materials or radioactive animals?

No

Co-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?

Co-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.

[redacted] has over [redacted] years of experience in a laboratory animal setting. [redacted] has experience in animal handling and anesthesia in the [redacted] Labs since [redacted] will be responsible for animal care, anesthesia, monitoring and euthanasia.

Has not worked with dogs previously. [redacted] will be trained/monitored by PI and DLAM staff.

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 coordinating the laboratory scheduling and will assist with laboratory procedures as necessary. [redacted] will be responsible for animal care, anesthesia monitoring, handling, transfer, and euthanasia.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-Investigator

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.

[redacted] is a [redacted] resident at [redacted] with extensive research background in translational research. [redacted] is adept with statistical methods used in biomedical research and image analysis including computer-based image processing such at MATlab).

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

Statistical analysis. Image processing. Data entry and interpretation. Manuscript and grant preparation.

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?

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.

experience in a laboratory animal setting. experience in animal handling and anesthesia in the [redacted] Labs.

Has not worked with dogs previously. [redacted] will be trained/monitored by PI and DLAM staff.

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 coordinating the laboratory scheduling and will assist with laboratory procedures as necessary. [redacted] will be responsible for animal care, anesthesia monitoring, handling, transfer, and euthanasia.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

DLAM Staff View Person Detail

Email:		**UID:**	999999998
Phone:	[redacted]	**Degree:**	
Fax:		**Dept:**	DIV LAB ANIMAL MEDICINE
Status:	Staff		

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 veternarian

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

Providing analgesia and anesthesia during survival surgery and imaging. Provide euthanasia. Monitor animal during surgery and imaging. Provide postoperative care.

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?

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.

experience in a laboratory animal setting. experience in animal handling and anesthesia in the [redacted] Labs.

Has not worked with dogs previously. [redacted] will be trained/monitored by PI and DLAM staff.

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

coordinating the laboratory scheduling and will assist with laboratory procedures as necessary. [redacted] will be

responsible for animal care, anesthesia monitoring, handling, transfer, and euthanasia.

Will this person handle radioactive materials or radioactive animals?

No

Contacts

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

Funding

1. Funding Types (Check All That Apply):

- ☐ Department
- ☑ 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:

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 has been changed to dog as the rabbit cerebral arteries are too small to accommodate endovascular sympathectomy devices. [redacted]

Rationale

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

1. Demonstrate the efficacy of endovascular sympathetic denervation to reduce vasospasm in the setting of subarachnoid hemorrhage.

2. Obtain preliminary data to apply for, and successfully achieve, external funding for device development and

larger preclinical trials.

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

Vasospasm is a significant cause of morbidity and mortality in people suffering from hemorrhagic stroke/aneurysm. The endovascular sympathectomy approach to treat this condition is novel, having never been tried before. Previous sympathectomy procedures have required open surgery, resulting in unacceptable morbidity. However, the approach is viable; endovascular sympathectomy has been performed with success in the human renal artery with a less than 1% complication rate. While morbid, prior cranial sympathectomy for cerebral vasospasm has been shown to reduce vasospasm. Information gained from these studies may lead to better treatment of cerebral vasospasm in humans suffering from aneurysmal subarachnoid hemorrhage. There are currently no effective treatments. Medications such as beta blockers are often ineffective in treating vasospasm. Many patients receiving medical management for vasospasm will still suffer strokes.

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.

a) Dog is most appropriate species for this study because it is the phylogenetically lowest species with a cerebral artery of appropriate size. The dog basilar artery measures 1.5-1.8 mm in diameter. The sympathectomy catheter is 1.33 mm in diameter. In comparison, the largest rabbit intracranial artery is less than 1 mm in diameter. Structural differences such as the rete mirabile makes pigs ineligible for this model.

b) Dog artery is more similar to human in its response to subarachnoid hemorrhage and its endothelial properties. Rabbit and pig species respond to subarachnoid hemorrhage in a dissimilar manner compared to humans and results may not be translatable. The most data on cerebral vasospasm in animal models is derived from dog experiments.

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. [redacted]
7. [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.

Blood vessel irritation will be caused in dogs using the standard experimental model of bleeding in the brain from a ruptured aneurysm, which involves injection of blood into the area around brain. Irritation is assessed with an angiogram, either by injecting contrast material through a tube in the artery (angiogram) or through a vein (CT angiogram and CT perfusion). This allows for numerical assessment of artery size and other important metrics. Control and treated animals are followed over time using a combination of CT and angiography to assess for changes in artery size, blood flow in the brain, blood volume in the brain and how long it takes for blood to enter and then leave the brain. Microscopic analysis is then performed to evaluate device related blood vessel injury and confirm burning of the nerves around the blood vessel.

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.

1. Subjects

10 mongrel dogs will be procured through an approved vendor. The canine model of aneurysmal subarachnoid hemorrhage (aSAH) is well-established, resulting in reproducible changes in arterial caliber and CT perfusion (CTP) metrics. The response to cervical sympathetic block has also been studied previously in models of aSAH and shown to result in cerebral vasodilation.

2. Inducing Vasospasm

Vasospasm will be induced according to the detailed description in the "Surgery" section.

3. Endovascular Sympathetic Denervation (ESD)

Endovascular Sympathetic Denervation will be performed according to the detailed description in the "Surgery" section.

4. Assessing Response to Endovascular Sympathetic Denervation

CT angiogram (CTA) and CTP [redacted] serve as adjunctive diagnostic tests. Images are processed on a separate workstation using Vitrea software for quantitative analysis of luminal diameter, cerebral blood flow (CBF), mean transit time (MTT), and cerebral blood volume (CBV).

CTA and CTP are performed on all dogs at baseline on day 1, prior to intracisternal injection of autologous blood. On day 4, CTA and CTP are repeated to assess the degree of vasospasm. This is followed by a catheter angiogram [redacted] before, and 10 minutes after, endovascular sympathectomy and sham procedure. Standard anteroposterior and lateral angiographic views are complemented with selective 3-dimensional angiography of spasmed vessels. Standard views are visually assessed to quantify arterial transit time on a frame-by-frame basis. 3D images are processed on a separate workstation for quantitative analysis of luminal diameter.
On day 5 repeat CTA, CTP and cerebral angiogram via left femoral artery access evaluate the response to ablation and sham. A final CTA and CTP are performed on day 7.

5. Histopathology

Following CTA and CTP on day 7, dogs remain anesthetized and are then euthanized in the [redacted] lab by DLAM staff. The thorax is opened and a cannula placed into the left ventricle. Subsequently, the descending aorta is clamped and the right atrium opened. Perfusion is performed with 500 mL phosphate buffered saline, followed by fixation with 500 mL 10% paraformaldehyde. The whole brain and cerebral arteries are removed and placed in 10% paraformaldehyde. Specimens are then handled by the [redacted] Arteries from both control and treated dogs are sectioned, stained and compared to evaluate for endothelial injury and thermal denervation.

6. Statistical Analysis

Based on prior studies of cerebral vasospasm using the 2-hemorrhage model, a 50% increase in basilar artery diameter following ESD would yield a power of 97% with a 5% significance level in a sample size of 5 treated and 5 control dogs. Change in arterial lumen diameter serves as the primary outcome; measurements from catheter angiography and CTA will be analyzed separately. Descriptive statistics such as means, standard deviations and 95% confidence interval estimates will be generated from luminal diameter, CBF, MTT and CBV variables at the various time points described above. Two sample t-tests and repeated measures analysis of variance will be performed at the 5% level of significance using SPSS statistical software.

7. References

[redacted]

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:	

Sex:	Male
Pain Category:	D
Previous Number of Animals Approved:	10
Change in Number of Animals Needed (+/-):	0
Number of Animals Needed for the 3 Year Period:	10

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
- [x] Failure to groom, unkempt appearance
- [x] Open sores/necrotic skin lesions
- [x] Loss of appetite
- [x] Weight loss.
  Percentage weight loss (max allowable 20%): 5
- [ ] Other:

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.

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 object of this study is to show biological responses of living tissue resulting from ESD treatment and the effects of surrounding peri-vascular structures. And since ESD induced alterations will involve the entire tissue microenvironment, these experiments cannot be performed using in vitro cell culture.

[redacted]

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

The expected level of pain induced by ESD is minimal, if any. ESD, unlike other ablation techniques, does not involve any incisions. Because the ESD device must be placed within the arterial lumen, vascular access is required. We do not expect significant tissue death or organ dysfunction, although transient local inflammation may occur at the target site.

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

According to the power analysis provided by our statistician, 10 animals is minimum number of subjects needed to achieve a significant result.

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
- [ ] 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

Keywords used:

dog and sympathetic denervation and alternative
dog and sympathetic denervation and reduce and distress
dog and sympathetic denervation and pain
dog and subarachnoid hemorrhage
dog and endovascular catheterization

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.

8/15/2016

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

1953-2016

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)?

No

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.

Criteria for premature termination of animals include any uncorrectable serious adverse effect as a result of the ESD
procedure. These effects are: severe pain or discomfort not controlled by pain medication, prolonged lethargy or

seizures, and unstoppable bleeding from the vascular access. Evaluation of the veterinarians at DLAM will always be requested and treatment recommendations followed, including premature termination. In the unlikely event of minor hematoma and pain from the vascular access, the animal would be treated with analgesia.

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.

7. Environmental Enrichment: UCLA 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.

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

NA - Animals are socially housed

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.

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	Research Area **Reason:** Intervent onal procedures (survival surgery), imaging including CT scan and angiography, and euthanasia.
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** Intervent onal procedures (survival surgery), imaging including CT scan and angiography, and euthanasia.
[redacted]	[redacted]	Dog	Surgery Area - Survival **Reason:** Intervent onal procedures (survival surgery), imaging including CT scan and angiography, and euthanasia.
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** [redacted] room w th fume hood for Formaldehyde use during perfus on following euthanasia.
[redacted]	[redacted]	Dog	[redacted]

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:	pharmaceutical-grade buprenorphine
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	0.02mg/Kg
Volume:	
Frequency:	pre-operative
Route:	sc
Length of treatment/administration:	Pre op and 72 after surgery
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	pharmaceutical-grade carprofen
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	4mg/kg
Volume:	
Frequency:	q24 hours pre and post procedure
Route:	sc
Length of treatment/administration:	Pre op and 72 after surgery
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	pharmaceutical grade dexmedetomidine
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	10mcg/kg
Volume:	
Frequency:	once per anesthesia event
Route:	im
Length of treatment/administration:	30 minutes
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pharmaceutical-grade isofluorane
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	1.5-2.5%
Volume:	
Frequency:	
Route:	inh
Length of treatment/administration:	1 hour
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pharmaceutical-grade Lidocaine
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	1 mg/Kg
Volume:	
Frequency:	once per anesthesia event
Route:	sc
Length of treatment/administration:	1 hour
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pharmaceutical-grade Midazolam
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	0.2 mg/Kg
Volume:	
Frequency:	once per anesthesia event
Route:	iv

Length of treatment/administration:	1 hour
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pharmaceutical-grade Propofol
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	4-8 mg/Kg
Volume:	
Frequency:	once per anesthesia event
Route:	iv
Length of treatment/administration:	1 hour
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	pharmaceutical-grade Omnipaque 300
Species:	Dog
Medication Type:	Other
Dose or Concentration:	
Volume:	10-50 ml
Frequency:	intraprocedural
Route:	other: ia
Length of treatment/administration:	1 hour
Purpose:	Other: imaging

Drug/Compound Name:	pharmaceutical-grade Omnipaque 350
Species:	Dog
Medication Type:	Other
Dose or Concentration:	
Volume:	50-100 ml
Frequency:	intraprocedural
Route:	iv
Length of treatment/administration:	for CT
Purpose:	Other: imaging

Drug/Compound Name:	pharmaceutical-grade Rocuronium
Species:	Dog
Medication Type:	Other
Dose or Concentration:	1-2 mg/Kg
Volume:	
Frequency:	repeated intraoperatively as needed during image acquisition
Route:	iv
Length of treatment/administration:	1 hour
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?**

   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.**

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

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

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

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 CO2 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:	veterinary grade pentobarb tol
Species:	Dog
Dose or Concentration:	100-200 mg/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:

- [x] Blood
- [x] Other Collected: brain

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

The brain will be excised following euthanasia for pathological studies.

Arterial blood will be collected immediately prior to intracisternal injections. A total of 2 intracisternal injections will be performed per animal.

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

entire brain

0.4 ml/kg of blood.

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

Following euthansia, the brain will be immediately excised using surgical instruments.

Arterial blood will be withdrawn from the femoral artery.

5. Describe how anemia and infection will be prevented.

Sterile technique for blood draw to prevent infection. The amount of blood withdrawn is not sufficient to produce anemia. The withdrawal site will be compressed manually to achieve hemostasis.

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
- [x] Fasting: 12 hours
- [ ] Other:

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.

Yes

State name of agent(s):

Rocuronium

Provide justification below.

See ARC Pol cy on Neuromuscular Blocking Agents.

Animal must not move during ESD. There are no alternative methods to ensure the animal will not move during ESD. If the animal moves during ESD, this may result in pain and injury to the animal.

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.

- [x] Respiration rate
- [x] Heart rate
- [ ] EEG
- [ ] EKG
- [x] 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.

water-circulating heading pads.

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: 1-2 hours

Non-Survival: 30 minutes to 1 hour

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.

In order to induce vasospasm using the standard, accepted 2 hemorrhage model of intracisternal injection of autologous blood, perform the proposed ESD treatment, and monitor its effectiveness with catheter angiography, multiple survival surgeries are necessary. Each animal will undergo 4 survival surgeries.

1. [redacted]

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

For surgical procedures animals will be premedicated with Midazolam, Buprenorphine and Carprofen, induced with Propofol and maintained with Isoflurane. Please refer to the medication section for specific dose and route.

5 dogs are randomized to diagnostic angiography control and 5 to angiography combined with endovascular sympathectomy.

1. Inducing Vasospasm

On day 1, dogs are sedated, anesthetized, and intubated by veterinary personnel. The dog is weighed. Heartrate, oxygen saturation, and rectal temperature are monitored throughout the procedure. Vasospasm is accomplished using the 2-hemorhhage model: The dog is placed prone on the CT table. A CT of the head is performed to identify the cisterna magna and plan the needle trajectory. A No. 22 needle is placed percutaneously into the cisterna magna, and 0.4 ml/kg of cerebrospinal fluid (CSF) is removed. An equal volume of fresh autologous arterial nonheparinized blood (drawn from the common femoral artery) is injected at a rate of 2 ml/min. The dogs are tilted with the tail up for 30 minutes to facilitate settling of the blood around the basilar artery by gravity. Intracisternal injection of autologous blood is repeated in the same manner on day 3.

2. Endovascular Sympathetic Denervation

On day 4, right femoral artery access is achieved. A cut down to isolate and expose the femoral artery with subsequent ligation of the vessel after sheath removal. For the cut down, a 1-2 cm incision is made over the vessel, the artery is isolated using blunt dissection, the vessel is tied distally and the sheath placed. Once the sheath is removed, the vessel is ligated and the incision closed in layers using vicryl, the skin is closed with an intradermal pattern. A 4-Fr catheter is navigated into the aortic arch. The catheter is positioned to sequentially evaluate the bilateral carotid and vertebrobasilar systems. Once the region of most severe spasm is determined, a selective 3-dimensional angiogram is obtained. Systemic heparinization is achieved and an endovascular sympathectomy catheter (Symplicity, Medtronic Inc.) is navigated into the spasmed arterial segment. If the vessel lumen cannot accommodate the ablation device, the device will be positioned at a feasible distance proximal to the stenosis.

The device is activated in 5 treated dogs and remains inactive in 5 controls. In the former, a radiofrequency pulse is applied to the endoluminal surface by an electrode at the distal catheter tip. Ablative treatment in performed in a helical fashion, from distal to proximal, during 1 catheter pass with approximately 5mm of longitudinal and rotational space between each ablated surface. After ablation, the tip of the catheter is straightened and removed and an angiographic run is performed to confirm the absence of dissection or thrombosis. Any endovascular complications are documented. The sheath and catheter are then removed and the arteriotomy closed by manual compression.

3. Assessing Response to Endovascular Sympathetic Denervation

A catheter angiogram is performed before, and 10 minutes after, endovascular sympathectomy and sham procedure. Standard anteroposterior and lateral angiographic views are complemented with selective 3-dimensional angiography of spasmed vessels. Standard views are visually assessed to quantify arterial transit time on a frame-by-frame basis. 3D images are processed on a separate workstation for quantitative analysis of luminal diameter.

On day 5 repeat cerebral angiogram via left femoral artery access is performed in the manner stated above to evaluate the response to ablation and sham. Again, a cut down to isolate and expose the femoral artery with subsequent ligation of the vessel after sheath removal. For the cut down, a 1-2 cm incision is made over the vessel, the artery is isolated using blunt dissection, the vessel is tied distally and the sheath placed. A 4-Fr catheter is navigated through the sheath into the aortic arch. The catheter is positioned to select the vertebrobasilar systems. A selective 3-dimensional angiogram is obtained. The catheter and sheath are then removed and the vessel is ligated and the incision closed in layers using vicryl, the skin is closed with an intradermal pattern.

4. Non-Survival Surgery

On day 7, dogs are anesthetized in the animal procedure room. After intravenous injection of phenobarbital, the thorax is opened and a cannula placed into the left ventricle. Subsequently, the descending aorta is clamped and the right atrium opened. Sterile instruments are used. Perfusion is performed with 500 mL phosphate buffered saline, followed by fixation with 500 mL 10% paraformaldehyde. The whole brain and cerebral arteries are removed and placed in 10% paraformaldehyde. Specimens are then handled by the [redacted]
Arteries from both control and treated rabbits are sectioned, stained and compared to evaluate for endothelial injury and thermal denervation.

5. References

[redacted]

[redacted]

11. Please indicate the suture materials to be used:

- [x] Internal: absorbable sutures (e.g., Dexon, Vicryl)
- [x] 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):

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.

thermo-regulation, cardiovascular and respiratory function, and post-operative pain or discomfort. Buprenorphine and Carprofen may be administered by the DLAM veterinarian as needed for analgesia. Please refer to the medication section for specific dose and route. Animals will be monitored after surgery until they are alert and ambulatory. A temperature probe will be used to assess temperature, and water-circulating heating pad used as needed.

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.

observed continuously by trained personnel during the immediate anesthetic-recovery period and daily after anesthetic recovery.

Species Surgery

Species:	Dog
Number of Animals:	10
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	4
Time Between Surgeries:	1-2 days

Species:	Dog
Number of Animals:	10
Surgery Type:	Nonsurvival Surgery
Surgeries per Animal:	1
Time Between Surgeries:	immediately after pr or survival surgery

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).

Assessing Response to Endovascular Sympathetic Denervation

CTA and CTP [redacted] serve as adjunctive diagnostic tests. Images are processed on a separate workstation using Vitrea software for quantitative analysis of luminal diameter, cerebral blood flow (CBF), mean transit time (MTT), and cerebral blood volume (CBV).

CTA and CTP are performed on all dogs at baseline on day 1, prior to intracisternal injection of autologous blood. On day 4, CTA and CTP are repeated to assess the degree of vasospasm.

On day 5 repeat CTA and CTP evaluate the response to ablation and sham. A final CTA and CTP are performed on day 7.

Dogs will be sedated and intubated for CT imaging by the DLAM veterinarian staff using Dexmedetomidine, followed by propofol and isoflurane. Please refer to Medication section for drug dosage and route. Then, dogs are placed prone

on the CT table. Iodinated contrast in injected intravenously and CT images are obtained. Following the CT, dogs are recovered by DLAM staff.

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

No clinical response is anticipated. Potential complications include allergic reaction to iodinated contrast agent, which would be managed by the DLAM vetereinarian.

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: charcoal canister in [redacted]

Scavenging Location

This section is empty.

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
Midazolam	IV	100-200 mg/kg	2-5 minutes	
Paraformaldehyde	tissue imersion	1L	post-euthanasia	

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] **12/3/2018**

FS Assurance

This section is empty.