UCI Office of Research
Research Management System

Animal Use Protocol (

Title

General Information

Version: 19.0

Project Overview

Provide a non-technical, lay-language summary of your project.
Uncheck this box to remove all text from the box below

Myocardial ischemia is the lack of blood flow (and hence oxygen supply) to the heart. If blood is not restored to the area, this leads to death of cardiac tissue (myocardial infarction). A common symptom of myocardial ischemia is cardiac angina (chest pain). Other symptoms include an increase in blood pressure and heart rate, the body's attempt to restore blood supply to the heart. The aim of this study is to determine the mediators and their receptor subtype responsible for stimulation of cardiac sensory nerves that signal pain during myocardial ischemia and the related reflex cardiovascular responses including hypertension and heart rate increase. This represents the afferent limb (sensory nerve fibers that send information to the central nervous system) of the cardiovascular reflex which results in an increase in heart rate and blood pressure. By knowing specifically which chemical mediators and their receptor subtypes are involved in the events preceding a heart attack, therapies and drugs can be developed to target these compounds and their receptor subtype and help combat symptoms of high blood pressure, chest pain, and heart attacks.

Study Characteristics
Surgery
Types of Surgery
Terminal (animals will not recover from anesthesia)
Major
Survival
Major
Will MULTIPLE major survival surgeries be performed on a SINGLE animal?
No
Paralytic Agents
Guidelines for the use of paralytic agents (neuromuscular blocking agent) in live animals:
Scientific Justification for Use of Paralytic Agents
Uncheck this box to remove all text from the box below

This study involves measuring the responses of the cardiac spinal afferent nerves and renal sympathetic nerves activity to ischemic mediators and myocardial ischemia. Thus, paralytic agents must be used to prevent the flexor movement activation of cardiac sympathetic nerve induced by non-cardiac-related stimuli.

You MUST provide details about the paralytic agents in other Protocol Tabs:

Project Continuation

Is this application a 3-year renewal of a previously approved protocol?

Yes

Total # of animals used in the last 3 years

Uncheck this box to remove all text from the box below

Species	USDA Pain Category *(B, C, D, or E)*	# of Animals used in last 3 years
Cat	D	0
Rat	D	0

Did any adverse events or unanticipated problems with animal health, behavior or well-being occurred during the last 3 year period of this study?

No

Progress Summary

Uncheck this box to remove all text from the box below

In this study stimulation of cardiac P2X2/3 and P2X3, but not P2Y receptors, with intrapericardial application of P2X receptor agonist powerfully evokes cardiac sympathoexcitatory reflex (CSR) responses through activation of cardiac spinal afferents and that endogenous opioids suppress the P2X receptor-mediated CSR responses.

A submitted paper in revision

Important notes: although we did not use any animals during last three years, a grant application with this animal protocol has been re-submitted and funding on pending now.

Justification for Continuing the Project

Uncheck this box to remove all text from the box below

Myocardial ischemia through actions of multiple ischemic metabolites activates cardiac spinal afferents and evokes cardiac sympathoexcitatory reflex responses

characterized by increases in sympathetic outflow, elevations in blood pressure and heart rate, and tachyarrhythmia. These reflex responses can be lethal in the setting of a heart attack or following bypass surgery. Although we have shown that several ischemic mediators in an interactive and multifactorial fashion stimulate cardiac spinal afferents, leading to generate cardiac sensory nerve input through the central cardiovascular regulation centers and in turn cause excitatory cardiovascular responses, the mechanisms underlying the ischemia-induced cardiac spinal afferent activation and the related cardiac sympathoexcitatory reflex responses remain unclear and represent our current investigation. this lon-term research project has been funded by the NIH since year . the current studies will continuously investigate the role of novel and important ischemic mediators in activation of cardiac afferents and reflex responses and the underlying mechanisms.

Funding Source	Funding Status	Award/Proposal #	Billing Account #

Funding & Billing Information

Other Funding Sources (not captured in table above)

Has this project undergone peer-review by an extramural sponsor or funding agency? (e.g., NIH study section)
Yes

N/A

PI Home Department
Uncheck this box to remove all text from the box below

Species

Cat
Rat

Cat

Species Justification

Provide scientific justification for why the proposed species is the most appropriate model for this research.
Uncheck this box to remove all text from the box below

A live vertebrate animal model is necessary to study reflex cardiovascular responses to the stimulation of cardiac sensory nerve endings. A fully connected cardiovascular and nervous system needs to be intact in order to observe the full affects and true nature of cardiac reflexes during myocardial ischemia. The pathway for these reflex responses involves both cardiovascular and nervous system. Thus non-animal models are not adequate for this study.

Other investigators, including ourselves, have demonstrated that the neural control of the cat and rat's cardiovascular system is similar to that of humans. Our previous studies have shown that it is possible to study cardiac reflexes induced by chemical stimulation in the rat and cat. We will use rats for studying the reflex responses to chemical stimulation, so as to minimize the number of cats used.

We will use cat for studying the reflexes to myocardial ischemia and afferent responses to cardiac stimulation because we only can study cardiac reflex and cardiac afferent nerve fibers during myocardial ischemia (i.e., reduced blood flow) in the cat by occluding a coronary artery on the surface of the heart. Smaller animal species such as rats and guinea pigs cannot be utilized for this part of the proposed study during myocardial ischemia because of the technical difficulties associated with 1) selective occlusion of coronary arteries that sub-serve the receptive field of the afferent under study, and 2) isolation and recording of cardiac sympathetic afferent nerve fibers. The hearts of rats and guinea pigs are simply too small to perform these surgical procedures successfully. With smaller hearts, occlusion of the coronary artery will reduce blood flow to a much larger area of the heart than desired. This could cause irreversible damage to cardiac muscle tissue and possible trauma to the overall health of the animal as a significant drop in blood pressure would immediately develop. We have had many successes in achieving coronary occlusion in the cat without inflicting damage. We have also been able to skillfully separate a single nerve fiber from the sympathetic chain (which contains a bundle of nerve fibers) and accurately record the nerve firing action potentials.

Animal Characteristics

List the specific strains that will be used.

Phenotypic Abnormalities or Special Health Conditions

Additional Information about Species/Strains

Rat

Species Justification

Provide scientific justification for why the proposed species is the most appropriate model for this research.
Uncheck this box to remove all text from the box below

Other investigators, including ourselves, have demonstrated that the neural control of the cat and rat's cardiovascular system is similar to that of humans. Our previous studies have shown that it is possible to study cardiac reflexes induced by chemical stimulation in the rat and cat. We will use rats for studying the reflex responses to chemical stimulation, so as to minimize the number of cats used.

Animal Characteristics

List the specific strains that will be used.

Phenotypic Abnormalities or Special Health Conditions

Additional Information about Species/Strains

Rationale & Alternatives

Search Results

Date of Search	Time period from	Time period to	Database	# of Results	Keywords
Aug-21-2019	Jan-01-1966	Aug-21-2019	Web of Science	2	Cardiac afferents, cardiac sensory nerves, myocardial ischemia, ischemically sensitive, dorsal root ganglion, cardiac stimulation, cardiovascular reflexes, cat alternative, rat alternative, cardiovascular models, in vivo recording, sympathetic afferent activity recording, , opioids receptors, and endocannabinoids
Aug-21-2019	Jan-01-1966	Aug-21-2019	PubMed	3	Cardiac afferents, cardiac sensory nerves, myocardial ischemia, ischemically sensitive, dorsal root ganglion, cardiac stimulation, cardiovascular reflexes, cat alternative, rat alternative, cardiovascular models, in vivo recording, sympathetic afferent activity recording, opioids receptors, and endocannabinoids

Database Searches

Discussion of Search Results
Uncheck this box to remove all text from the box below

We are investigating cardiovascular responses to stimulation of cardiac sympathetic sensory nerves. We have published well over 30 studies and always strive to reduce the number of animals and to refine the experimental design and procedures. As suggested, we have conducted literature search again. However, there is no similar study to ours, which has been reported before. In this regard, there is no alternative protocol to replace the survival protocol to identify opioids receptors and endocannabinoids (ECs) receptors in the cardiac sensory neurons located in the dorsal root ganglia. Thus, our study is not an unnecessary duplicate of any other previous work.

Other Sources Used to Consider Alternatives

Replacement

Discuss efforts to partially or fully replace live animals with in vitro models, (i.e. cell culture), computer simulation, or use of a less-sentient species (e.g. insects).
Uncheck this box to remove all text from the box below

We are always undertaking extensive research into the development of other non-animal models that would allow us to substitute the utilization of live animals for this project. However, there is no in vitro, model that allows us to study reflex cardiovascular responses to nerve fiber stimulation. Moreover, we are studying reflex responses and sensory nerve discharge activity in intact and complex systems such that our endpoint measures cannot be collected from computational models or non-mammalian systems.

Reduction

Describe the steps you have taken to reduce the number of animals to the minimum required to obtain scientifically valid data.
Uncheck this box to remove all text from the box below

The literature search does not help significantly with reducing numbers of animals, c in respect with studying the mechanisms of the cardiovascular reflexes evoked by stimulation of cardiac sympathetic afferents. In order to reduce the number of animals used, more than one protocol is performed on each animal. We will use minimum of animals (n=3/group) for the survival protocols. By performing on a fewer number of animals for the survival study, we will minimize the number of surgeries performed and reduce the animal use count.

Refinement

Explain how the experimental design and procedures have been refined to improve efficiency and minimize pain and distress.
Uncheck this box to remove all text from the box below

We are constantly refining our experimental procedures to limit the surgical time and the extensiveness of the surgery. We also continually refining our techniques such as surgical skill and delivering appropriate anesthetics into animal and hence enhancing animal well-being.

The length of ischemia is a crucial factor that leads to ventricular fibrillation and hence death. Shorter ischemia could lead to lower death rate. In this project we choose to use 5 min of myocardial ischemia We also will use the most short and reasonable period of myocardial ischemia in the ischemic model to lower death rate. There are reasons for this duration; first, previous clinical studies have demonstrated that angina (i.e. acute myocardial ischemia) typically lasts for several minutes to < 20 minutes in patients

Second, experimental studies have demonstrated that occlusion of a coronary artery for 5 to 20 min (i.e., myocardial ischemia) induces a reversible ischemic injury and produced sufficient ischemic metabolites that simulate angina in patients very well

. We have used the 5 min of myocardial ischemic model in many of our previous studies (

. Thus we believe that 5 min is most short and reasonable duration and will use the 5 min of ischemia as the ischemic model in the present project, in which we could test our working hypotheses with justified pathological reason and this duration could cause less mortality. We have addressed brief 5 min of ischemia in Section 3, on pages 14 and 16.

Experts have trained each member and will train the new member of the laboratory on the techniques they are using, and each member is independently vigilant for new innovations with regarding the techniques and procedures that are used in this project. As such, we always employ the most refined experimental protocols possible to further improve the overall success rate. In addition, we invite outside investigators to our laboratory and we send our staff to other laboratories to learn the latest techniques that might apply to our studies.

Study Segments

Experimental Design	Species
Study Segment	Cat,Rat

Study Segment

Species

Cat
Rat

Experimental Design Summary

Does this study segment tab describe the establishment and maintenance of a breeding colony?

Provide a concise description of the experimental design, describing all experiments to be performed.
Uncheck this box to remove all text from the box below

General Rationale: During myocardial ischemia, multiple ischemic metabolites activate and sensitize sympathetic (spinal) afferents, which lead to angina pectoris and excitatory cardiovascular reflex responses, some of which can be lethal in the setting of a heart attack or following bypass surgery. The mechanisms underlying ischemia-mediated cardiac afferent activation and the related cardiovascular reflex responses are unknown and represent our current investigation. Our research team is the only laboratory in the world studying the activation mechanisms of cardiac sympathetic afferents and the associated cardiovascular reflexes. This long-term research project has been funded by the NIH since . During funding of this grant, research and review papers have been published in this specific area of study. Our investigations have shown that a number of ischemic metabolites in an interactive and multifactorial fashion stimulate cardiac afferents and evoke excitatory cardiovascular reflex responses. The current studies (1-3) investigate the role of novel and important ischemic metabolites, including in activation of cardiac afferents and reflex responses. For these metabolites there are no data in the literature that speak to their importance, although studies by others indicate

these metabolic factors are produced during ischemia and have the potential to contribute to activation of these important nerve endings. We will observe their individual and interactive roles in stimulating cardiac sensory nerves that are known to cause excitatory reflex cardiovascular responses during myocardial ischemia and reperfusion. Studies of interactions between the mediators are important since they are released simultaneously and can interact, leading to a net sensory neural response that shares some components but is different from individual actions of each mediator on the nerve endings in the heart.

Note: The neural control of cardiovascular system is similar in cats and rats. Previous studies from us and others have shown that it is possible to study cardiac reflexes induced by chemical stimulation in rats. However, we only can study cardiac reflex and cardiac afferent nerve fibers during myocardial ischemia (i.e., reduced blood flow) in cats by occluding a coronary artery on the surface of the heart. Smaller animal species such as rats and guinea pigs cannot be utilized for the proposed study during myocardial ischemia because of the technical difficulties associated with 1) selective occlusion of coronary arteries that sub-serve the receptive field of the afferent under study, and 2) isolation and recording single-unit activity of cardiac sympathetic afferent nerve fibers. The hearts of rats and guinea pigs are simply too small to perform these surgical procedures successfully. With smaller hearts, occlusion of the coronary artery will reduce blood flow to a much larger area of the heart than desired. This could cause irreversible damage to cardiac muscle tissue and possible trauma to the overall health of the animal as a significant drop in blood pressure would immediately develop. As such, rat and cat will be used based on experimental approaches as described in the following experimental protocols.

Study #1

This study will determine the contribution of [redacted] to activation of cardiac sympathetic afferents by measuring single-unit nerve activity, impulse/sec and associated cardiovascular reflex responses including arterial blood pressure, heart rate and sympathetic nerve activity.

Rationale: Concentrations of [redacted] in coronary heart diseases or during myocardial ischemia are increased through overproduction and release from mast cell

and endothelial cells. [redacted] or agonists excite jejunal afferents and trigeminal nociceptive neurons and upregulate the excitability of vagal pulmonary sensory neurons through [redacted] However, the role of [redacted] in excitation of visceral afferents in any organ during ischemia is unknown. A model of regional myocardial ischemia will be used and the following ten groups evaluated:

Experimental protocols (please refer to Procedures Tab for surgical procedures in detail)

For terminal experiments, Study #1, a) – i), timelines and sequences of events will be: 1) cats will be anesthetized using ketamine and alpha-chloralose; 2) as described in detail in each protocol below, cardiovascular reflexes or cardiac sympathetic nerve activity will be studied during brief myocardial ischemia for 5 min and/or chemical stimulation of the heart with [redacted] before and after their antagonists, and experimental drugs will be administered; 3) animal will be euthanized at the end of each experiment.

a) Regional myocardial ischemia will be induced by coronary artery occlusion. Cardiovascular reflex responses to repeated coronary ischemia before and after administration of vehicle will be measured to examine repeatability and consistency of the reflex responses. This group will serve as a time control.

b) Cardiovascular reflex responses to recurrent coronary ischemia before and after administration of [redacted] will be measured. This protocol will determine if [redacted] the ischemia-mediated cardiovascular reflex responses.

c) As a control group, cardiovascular reflex responses to repeated application of exogenous [redacted] on the heart before and after administration of the vehicle will be measured to evaluate reproducibility of reflex responses to [redacted] agonist.

d) We will measure cardiovascular reflex responses to repeated epicardial application of [redacted]

e) Cardiac sympathetic afferents responsive to ischemia will be identified. The afferent responses to application of [redacted] be measured to examine if these agonists stimulate this group of cardiac afferents.

f) After identification of an ischemically sensitive cardiac sympathetic afferent, the afferent responses to [redacted] At last we will epicardially apply bradykinin to determine responsiveness of these afferents.

g) For another control group, cardiac afferent responses to [redacted] before and after administration of vehicle will be measured to evaluate the reproducibility of cardiac afferent responses to [redacted]

h) Cardiac afferent responses to repeat myocardial ischemia before and after [redacted] attenuates the afferent responses to ischemia.

i) We will examine the afferent responses to recurrent ischemia before and after the vehicle to examine repeatability of the afferent responses to ischemia. This group will serve as a time control.

j) Since it is impossible to detect specific sensory receptors located in the nerve terminals distributed in the heart, identification of their presences in the cell bodies, dorsal root ganglion (DRG) is considered as an alternative method. Thus, in this study, we will inject retrograde tracer dye (DiI, 1.7 mg in 0. 1 ml) into the pericardial space through the pericardium. The DiI tracer will be taken by nerve endings located in the heart and then will be transported backwardly to their cell bodies in the DRG. We will identify cardiac sensitive neurons containing [redacted] exist in sensory nerve originated from the heart. It usually takes 8-9 weeks for the DiI tracer to travel from nerve terminals to cell bodies in cat. Thus, a survival procedure is needed for this experiment.

For this survival experiment, a timeline and sequence of events will be:

1. Cats will be anesthetized using Ketamine/Midazolam and isoflurane subsequently.
2. The DiI will be injected into the cardiac muscle
3. The cat will be recovered from the surgical procedure

4. Nine to ten weeks later, the cat will be anesthetized with ketamine and alpha-chloralose. After animal euthanizing the animal, cardiac perfusion will be performed and DRG harvested.

Alternative protocols: It is possible that [redacted] may not play a role in these afferent and reflex responses in study #1. In this case, we will examine the role of [redacted] in sensitizing cardiac afferents and the cardiac reflex responses.

Abbreviations:

[redacted]

Study #1: Our current success rate in cardiac afferent recording studies is approximately 70% and for cardiovascular reflex study is approximately also 70% in cats.

Study #1(a-d): cardiovascular reflex study: 12 cats / 70% = 17 cats total for each group

4 groups x 17 = 68 cats needed;

Study #1(e-i): cardiac afferent study: 12 cats / 70% = 17 cats total for each group

5 groups x 17 = 85 cats needed;

Study #1j: anatomic study: 1 group x 3 cats = 3 cats.

Thus, for study #1 156 (68 + 85 + 3) cats total needed.

Study #2

This study will determine 1) contribution of P2 receptors and histamine to generation of cardiac reflexes responses and 2) interactions between and α,β-meATP in their modulation of cardiac sympathoexcitatory reflex responses including blood pressure, heart rate and renal sympathetic nerve activity in rats.

Rationale: myocardial ischemia evokes excitatory cardiac cardiovascular reflexes ( ) and that a number of ischemic mediators are produced during myocardial ischemia. However, the contributions or roles of the ischemic mediators including ATP, ADP and histamine in the ischemia-induced cardiac cardiovascualr reflex responses are unclear. ATP is P2 receptors agonist. P2 receptors include P2X and P2Y subgroups. α,β-meATP is P2X receptor agonist and ADP is P2Y agonist. Therefore, by knowing specifically which chemical mediators are involved in the events preceding a heart attack and the associated reflex response, therapies and drugs can be developed to target these compounds and help combat symptoms of high blood pressure, chest pain, and heart attacks. We observed that intrapericardial applictation of 40 µl of α,β-meATP (1.65 to 6.2 mM) evoked excitatory cardiovascular responses in rats. The α,β-meATP-evoked reflex responses were attenuated after application of . Thus, we will use rat model 1) to evaluate the contribution of P2 receptors including P2X and P2Y subgroup in cardiac reflexes responses and cardiac reflexes to histamine and trypsin as well as 2) to test the interactions between and α,β-meATP.

10 groups will be studied in Study #2.

Experimental protocols (please refer to Procedures Tab for surgical procedures in detail)

For terminal experiments, Study #2, a) – j), timelines and sequences of events will be:

1. Rats will be anesthetized using ketamine and alpha-chloralose;
2. As described in detail in each protocol below, cardiovascular reflexes will be studied during chemical stimulation of the heart with α,β-meATP, and experimental drugs will be administered;
3. Rat will be euthanized at the end of each experiment.

a) We will record cardiac reflex responses to intrapericardial ATP, α,β-meATP (3.2 mM), ADP (25-100 mM), histamine (1-10 mM), trypsin (125-500 μg) and saline in random order every 30 min.

b) We will study cardiac reflex responses to intrapericardial different doses of α,β-meATP (1.65 to 6.2 mM) to evaluate the dose-responses of cardiac reflex to α,β-meATP.

c) We will test the responses of cardiac reflex to intrapericardial α,β-meATP (3.2 mM) before and after administration of P2X antagonist TNP-ATP (50-120 μmol/kg) or P2X3 receptor antagonist RO-3 (50-120 μmol/kg) to evaluate the role of P2X receptors in the reflex responses to α,β-meATP.

d) As a time control, responses of the cardiac reflex to intrapericardial α,β-meATP (3.2 mM) before and after administration of saline will be measured to evaluate the reproducibility of cardiac reflex responses to α,β-meATP.

e) We will study the responses of cardiac reflex to intrapericardial application of α,β-meATP (3.2 mM) before and after administration of the modulates cardiac reflex responses to α,β-meATP.

f) We will investigate the responses of the cardiac reflex to intrapericardial α,β-meATP before and after intrapericardial application of the facilitates cardiac reflex responses to α,β-meATP.

g) We will examine reflex responses to intrapericardial application of α,β-meATP (3.2 mM) before and after application of the modulates reflex responses to α,β-meATP.

h) Cardiac reflex responses to intrapericardial α,β-meATP before and after administration of boosts cardiac reflex responses to α,β-meATP.

i) Cardiac reflex responses to intrapericardial α,β-meATP before and after administration of will be studied to determine

if boosts cardiac reflex responses to α,β-meATP.

j) Cardiac reflex responses to intrapericardial α,β-meATP before and after 2% procaine (80 μl) will be measured to examine the influence of local blockade of cardiac sensory nerves neurotransmission on cardiac reflex responses to α,β-meATP.

Study #2 (a-j): Our current success rate for this reflex activity study is approximately 65% in rats.

12 rats / 65% = 18 rats for each group

10 groups x 18 rats = 180 rats needed.

Thus, for study #2 180 rats total needed.

Study #3

This study will determine if the responses of the afferents to brief ischemia (5 min), and extracellular ATP to modulate cardiac afferent activity during ischemia.

Rationale: Myocardial ischemia leads to production and simultaneous release of not just excitatory chemicals but alsc and ATP partially share the G protein-coupled receptor (GPCR) signaling pathways. Previous studies have shown that somatic sensory nerve fibers activity induced by chemical and stimulation, which suggests that the activity of cardiac afferents activity during ischemia. Thus, during ischemia may modulate response of cardiac afferent to ischemia and other excitatory mediators. However, there is no data on the interactions between the excitatory mediators with respect to activation of cardiac sensory nerve endings during ischemia. Study #3 therefore will focus on and ATP on activation of ischemically sensitive cardiac sympathetic afferents. Eight groups will be studied during myocardial ischemia to identify ischemia sensitive cardiac sympathetic afferents:

Experimental protocols (please refer to Procedures Tab for surgical procedures in detail)

For terminal experiments, Study #3, a) – h), timelines and sequences of events will be:

1. Cats will be anesthetized using ketamine and alpha-chloralose;
2. As described in detail in each protocol below, cardiac sympathetic nerve activity will be studied during chemical stimulation of the heart with ATP, and experimental drugs will be administered;
3. Animal will be euthanized at the end of each experiment.

a) Cardiac afferent responses to repeated ischemia before and after administration of [redacted] antagonism enhances the responses of cardiac afferents to ischemia.

b) As a control group, responses of cardiac afferents to repeated ischemia before and after saline (2 ml, iv) will be measured to evaluate the reproducibility of the afferent responses to ischemia.

c) We will study the responses of cardiac afferents to epicardial application of ATP (2 µmol) before and after administration of a [redacted] modulates cardiac afferent responses to ATP.

d) As a time control, responses of the cardiac afferents to epicardial ATP before and after administration of saline will be measured to evaluate the reproducibility of cardiac afferent responses to ATP.

e) We will investigate the responses of the cardiac afferents to epicardial ATP before and after injection of the [redacted] enhances afferent responses to ATP.

f) We will examine afferent responses to epicardial application of [redacted] modulates cardiac afferent responses to trypsin.

g) Cardiac afferent responses to epicardial [redacted] will be studied to determine if [redacted] boosts cardiac afferent responses to [redacted]

h) As a control, afferent responses to epicardial trypsin before and after saline will be measured to examine the reproducibility of cardiac afferent responses

Alternative protocols: It is possible that may not interact with and extracellular ATP in these afferent. We will investigate the role of in the interactions between and these excitatory mediators.

Abbreviations:

Study #3 (a-f): Our current success rate for this afferent activity study is approximately 70% in cats.

12 cats / 70% = 17 cats total for each group

8 groups x 20 = 136 cats needed.

All protocols involve terminal procedures except for Study 1j which involve both survival and terminal components.

* Study 1j is a survival study protocol and only requires 3 animals because multiple ganglia can be collected from each animal for staining. The timeline for the survival procedures in Study 1j includes injection of tracer dye will be ten weeks followed by re-anesthesia with ketamine and α-chloralose (100 mg/kg, iv) to induce deep anesthesia, transcardial perfusion with saline and cold 4% paraformaldehyde in phosphate buffer, which also leads to euthanasia. Subsequently, the dorsal root ganglia will be harvested.

Animal Monitoring

Species	Monitoring Parameters	Monitoring Frequency	Responsible Person
Cat	Post-OP pain signs	3 hr in first 2 days	
Cat	Post-OP pain signs	Twice/day in rest d	
Cat	Post-OP infection si	3 hr in first 2 days	
Cat	Post-OP infection si	Twice/day in rest d	

Animal Monitoring Details

Clinical Signs or Symptoms of Pain/Distress
Uncheck this box to remove all text from the box below

Survival protocol: Post-Op: This survival procedure for labeling dorsal root ganglia has been used in our research since 2007, and the experience and success of using this preparation is illustrated by the following publications . Although we have not seen any problem in the previous studies, infection of surgical wound may occur after surgery in animal. Clinical signs of wound infections may include pus draining from the wound, redness, hot to touch, licking or scratching surgical wound. The animal with wound infection also may have loss of appetite, weight loss, and lethargy.

Terminal protocol: During brief 5 min of myocardial ischemia, cardiac arrest or fibrillations may occur by monitoring tachyarrhythmias, heart beating stop and sudden decrease in arterial blood pressure. We always monitor the abovementioned signs of animal when we induce myocardial ischemia in experimental animal. During ischemia, if we observe the occurrence of cardiac arrest or fibrillations, we will defibrillate and jumpstart the heart by using defibrillator with 1 to 3 joules/kg immediately, and make every attempt such as intravenously injecting lidocaine (3-4 mg/kg) to revive the animal.

Management Plan for Animal Monitoring
Uncheck this box to remove all text from the box below

Survival protocol: The body temperature should drop slightly indicating the animal is under anesthesia. Heart rate and blood pressure should be stable. Changes would indication some pain or distress in animal. Palpebral reflex test and muscle tone will also be used to assess level of anesthesia and used to assess pain in animals.

Monitoring of the animal will be continuous and Palpebral reflex test will be performed

every 20 to 30 minutes.

Post-operative care: We will monitor for abnormal animal behavior such as licking or scratching wound, appetite changes, alertness, and signs of infection (swelling, redness, or discharge).

Analgesic is given every 8-12 hours. Clinical signs of abnormal behavior or infection (as mentioned above) will be monitored. The animal will be monitored every 2-3 hours during the first two days. The following days, the animal will be monitored 2 times a day until the wound is healed (about 3-5 days). If any of the above signs of infection occur, we will consult with ULAR Veterinary Services regarding administration of the appropriate antibiotics, such as an ampicillin (8 mg/kg, SC). If further complications develop or if the animal's pain or distress cannot be alleviated, consultation with ULAR Veterinarian Services will take place to determine if the animal needs to be humanely euthanized.

Terminal protocol: Sedation is assessed through stability of heart rate and blood pressure. Change in stability of heart rate, blood pressure and core body temperature may indicate pain or distress. Response to corneal probing and muscle movement or trembling will also indicate that the animal is coming out of anesthesia. Lastly irregular respiration indicates animal is beginning to coming out of anesthesia. Supplemental anesthesia (please see section 7 for dosage) is given as needed. In addition, anesthetized animals have respiratory pattern that follow the ventilator rate. If struggling occurs anesthesia will be administered.

Arterial blood gases are performed throughout the experiment. Blood gas analysis reports provide information such as oxygen and carbon dioxide saturation levels in the blood which gives status of the animals' physiological condition and respiratory function. Blood gas analysis reports will also be used to prevent animal from falling into states of acidosis (having low pH) or alkalosis (having high pH). Based on our experience the acidosis is caused by metabolic and/or respiratory problem, in such case, we will correct the acidosis by administering 0.5 ml/kg of 8% NaHCO3 (IV), or by adjusting the respirator rate or tide volume. The alkalosis usually is caused by respiratory problem; in this case, we will correct the alkalosis by adjusting the respirator rate or tide volume. Assessment of adequate depth of anesthesia will be throughout the experiment as described above. In addition, the animals constantly are being monitored for stable heart rate and blood pressure. Supplemental anesthesia is given as needed according to details described above for level of sedation. During ischemia, if we observe the occurrence of cardiac arrest or fibrillations, we will defibrillate and jumpstart the heart by using defibrillator

with 1 to 3 joules/kg immediately, and make every attempt such as intravenously injecting lidocaine (3-4 mg/kg) to revive the animal.

In addition, blood gas analyses are checked periodically (every 30min – 1hr) to maintain animal homeostasis. Monitoring heart rate, blood pressure and assessment of anesthesia is continuous, particularly intensively watching these signals during performing myocardial ischemia procedure

Documentation of Animal Monitoring
Post-op monitoring log
Sample log templates are available on the IACUC website.
Other

Survival Post-operative Care: During the animal's recovery and housing period, we will record animal's behavioral signs indicating pain or distress, surgical wound infection signs such as any discharge, especially if thick or colored, abnormal warmth, redness, or swelling around the surgical site, or elevated body temperature as well as the treatments that have been given to animal by the researchers. The body temperature will be monitored 1-2 times/day If there is sign of wound infection until the infection be healed. Body weight will be measured at least 1 time/week for the first two week then 1 time/2-3 week for the rest weeks. All information will be written on the post-Procedural Monitoring Record sheet and kept in the animal protocol folder in the animal room or our laboratory for checking.

Terminal protocol: We will document all information regarding the blood gas, blood pressure and heart rate, body temperature data on the Surgical and Anesthetic Record sheet during the terminal procedure. Also we will record the amounts of anesthetic, 8% bicarbonates, and dextran that have been administered by the researchers on this sheet. The information will be stored in the Surgical and Anesthetic Record sheet folder and kept in our laboratory for ULAR veterinary staff and IACUC members checking.

Endpoints

Experimental Endpoints
Uncheck this box to remove all text from the box below

Experimental endpoints will be right after completion of experimental procedures.

Humane Endpoints
Uncheck this box to remove all text from the box below

Survival protocol: If animal become very severely ill because of the unsuccessful treatment of the infection of surgical wound and the animal's pain or distress cannot be alleviated during post-operative care, consultation with ULAR Veterinarian Services will take place to determine if the animal needs to be humanely euthanized and thus removed from the study.

Terminal protocol: Myocardial ischemia may result in lethal arrhythmias. Animals will be removed from study and euthanized if our treatment of lethal arrhythmias proves to be unsuccessful and prognosis of recovery is poor. Such conditions include cardiac arrest, severe blood loss, and/or respiratory failure.

Euthanasia

Will animals be euthanized at the end of the experiments?
Yes

Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply
Injectable Anesthetic Overdose

Species *(receiving the drug/agent)*	Drug/Agent	Dose Range *(mg/kg body wt)*	Route *(SQ, IP, IV, IM, etc.)*
Rat	Pentobarbital	150 – 200 mg/kg	IV
Cat	Pentobarbital	150 – 200 mg/kg	IV

Confirmation of Death in Animals
Open chest inspection of the heart
Exsanguination (cutting a major blood vessel)
Other

Rat, Cat: Intravenous injection of 1 M Potassium chloride (1 ml/kg) into animal when the animal is under deep general anesthesia.

You MUST...

Animal number calculation for experimental part Study Segment

Cat		
	Max	Description
	292	cats (Category D)
	292	**Cat**

Rat		
	Max	Description
	180	rats (Category D)
	180	**Rat**

Copy IACUC SOP templates

Procedure Descriptions

Survival Protocol

Anesthesia Induction

Pre-anesthesia will be induced with injection of Ketamine/Midazolam subcutaneously. The cat will be intubated with a cuffed endotracheal tube and connected to an artificial ventilator and anesthesia machine. Anesthesia is induced and maintained through inhalation of Isoflurane in 100% oxygen.

Following initial anesthesia, the animal will not be able to stand on its own, become unresponsive and the body will be limp. After intubation, the cat will be connected to an anesthesia machine and anesthesia is induced with Isoflurane. Level of anesthesia is adequate to begin surgical procedure when muscle tone is relaxed and heart rate drops slightly. Surgical plane of anesthesia will be confirmed.

Monitoring of Animal during Procedure

Surgical plane of anesthesia will be confirmed. During the surgical procedures, body temperature, monitored with a rectal probe, will be maintained at 37.5 to 39 °C with a

circulating heating pad. Blood oxygenation and heart rate will be monitored using a pulse oximeter. Response to corneal probing and muscle movement or trembling will also indicate that the animal is coming out of anesthesia at which point rate of Isoflurane inhalation is adjusted. Monitoring of the animal will be continuous and corneal probing will be performed every 15 minutes. Pre-surgical procedures, buprenorphine (0.005 – 0.01 mg/kg, i.m) will be administered and bupivacaine (2 mg/kg, SC) will be injected locally into the incision area. During post-operative care following the survival surgeries, we will administer the analgesic, buprenorphine (0.005 – 0.01 mg/kg, i.m) every 8-12 hours.

Artificial Ventilation

Cats will be ventilated at rate of 200-250 ml/kg/min at tidal volumes of 10ml/kg.

Aseptic Techniques

Survival procedure will take place in designated surgical suite in vivarium.

Surgeon and assistant will scrub hands and forearms with a disinfectant soap for three minutes and dry with a sterile towel. They will wear sterile surgical gloves, gowns, caps, surgical face masks, and shoe covers.

Animal preparation will include shaving the surgical area and the skin cleaned and disinfected with a chlorhexidine or povidone iodine-based disinfectant.

Surgical instruments will be sterilized by treatment in an autoclave.

Surgical Procedure

Cats will be prepared for surgery in the surgical suite located in the vivarium. Pre-anesthesia will be induced with injection of Ketamine/Midazolam subcutaneously. The surgical site will be shaved and disinfected. The cat will be intubated with a cuffed endotracheal tube and connected to an artificial ventilator and anesthesia machine. Anesthesia is induced and maintained through inhalation of Isoflurane in 100% oxygen. The eyes will be lubricated with a sterile ophthalmic ointment to prevent corneal drying. During the surgical procedures,

body temperature, monitored with a rectal probe, will be maintained at 37°C with a circulating heating pad and a heat lamp. A pulse oximeter will be clipped onto the tongue to monitor blood oxygenation and heart rate. Monitoring of heart rate and anesthesia levels will be continuous and corneal probing will be performed every 15 minutes. Amoxicillin (10-20 mg/kg, SC) will be administrated.

A one inch incision will be made on the left lateral thoracic region of the chest overlying the intercostal space between the fifth and sixth ribs. The location for pericardial incision will be confirmed by palpating the cardiac motion through the chest wall prior to making the incision. The underlying tissues will be carefully dissected to reveal the thoracic cavity. Once in the thoracic cavity, the thymus will be carefully retracted to expose the anterior surface of the pericardium sheath surrounding the heart. The pericardium will be puckered by a surgical hemostat and elevated. A 50 microliter Hamilton syringe will be inserted into the ventricular wall for injection of tracer dye. Prior to injection, the syringe will be withdrawn to draw in serous fluid from the pericardial space. This will confirm the placement of the syringe and no advancements will be made. 25 microliters of a suspension of in saline solution will be slowly (0.5 µl/s) injected into the cardiac muscle. Shortly after injection, the surgeon will begin to close the wound. The air will be suctioned out of the chest before the chest wound closure. The thoracotomy will be closed using non-absorbable suture which will be removed when the skin has healed completely (10-14 days). This is the end of the survival procedure. It will take approximately three hours to finish this surgical preparation. The animal will be placed into post operative care to recover.

Methods to Prevent Dehydration & Hypothermia

The eyes will be lubricated with a sterile ophthalmic ointment to prevent corneal drying. Body temperature will be monitored with a rectal probe. During the surgical procedures, body temperature will be maintained at 37oC with a circulating heating pad. Blood oxygenation and heart rate will be monitored using a pulse oximeter. Intravenous drip of 0.9% saline will be administrated at an initial rate of 10 ml/kg/hr as needed according to blood pressure during survival surgery.

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Post-Operative Care & Analgesic Usage

Before the cat awakens from surgery, we will administer the analgesic, buprenorphine

(0.005-0.01 mg/kg. im, please refer to section 7 for exact dosages) every 8-12 hours. The cat will be placed in a heated recovery cage with food and water in the vivarium and allowed to recover under close supervision.

We will administer the analgesic, buprenorphine every 8-12 hours during the next 48-72 hour period. Once the cat has fully awaken from anesthesia (about 6-12hrs after end of surgery), the animal will be transferred into a single housing cage in the vivarium. The animal will be monitored every 2-3 hours during the first two days. The following days, the animal will be monitored 2 times a day until the wound is healed. Animals usually recover within 3-5 days. A record of the cat's post-procedural care and monitoring will be kept as shown on the attached form. Terminal surgery will take place following a 10 weeks recovery period.

Terminal Protocol

Anesthesia Induction

Initial anesthesia is induced by subcutaneous or muscular injection of Ketamine/Midazolam. Anesthesia (alpha-chloralose) is given intravenously. Thereafter anesthesia (alpha-chloralose) is given intravenously prior to cannulation procedures with butterfly catheter into the brachial vein. Supplemental anesthesia (alpha-chloralose) is also given intravenously.

Surgical plane of anesthesia will be confirmed. Sedation is assessed through stability of heart rate and blood pressure. Change in stability of heart rate, blood pressure and core body temperature may indicate animal coming out of anesthesia. Response to corneal probing and muscle movement or trembling will also indicate that the animal is coming out of anesthesia. Experimental procedures take place after determining the adequate level of anesthesia. In addition, abnormal respiration and inconsistency in breathing with ventilator also indicate animal is not fully anesthetized.

Monitoring of Animal during Procedure

Surgical plane of anesthesia will be confirmed and maintained throughout the procedure. A rectal probe is inserted to monitor the animal's body temperature and is maintained with a heating pad and/or heat lamp. In addition, the animals constantly are being monitored for stable heart rate and blood pressure. Blood pressure will be monitored by pressure transducer, and heart rate is derived from the blood pressure by the device. Blood gases will

be measured by ABL5 blood gas machine. Response to corneal probing and muscle movement or trembling will also indicate that the animal is coming out of anesthesia at which point supplemental anesthesia (alpha-chloralose) will be given. Please refer to section 7 for exact dosages. In addition blood pressure and heart rate fluctuations will indicate that anesthesia is wearing off. In addition, abnormal respiration and inconsistency in breathing with ventilator also indicate animal is not fully anesthetized. After administration of paralytic agent, additional administrations of alpha-chloralose (10-20 mg/kg, iv) will be given every 30 min to maintain an adequate depth of anesthesia that will be assessed by observing the absence of conjunctive and corneal reflexes throughout the study.

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Artificial Ventilation

Cats will be ventilated at rate of 200-250 ml/kg/min at tidal volumes of 10ml/kg. Rats will be ventilated at rates of 80-90 breaths/min at tidal volumes of 6 ml/kg.

Aseptic Techniques

A clean surgical table is designated for surgery.

Clean surgical scrubs and gloves will be worn.

Animal preparation will include having all surgical sites will be shaved.

Clean surgical instruments will be used.

Surgical Procedure

Initial anesthesia is induced by subcutaneous injection of Ketamine/Midazolam. Anesthesia will be administered intravenously with butterfly catheter into the brachial vein. A femoral artery and vein will be cannulated via a small incision over the femoral artery and vein, which then are isolated to allow insertion of the cannulae for measurement of arterial pressure or administration of drugs and fluid, respectively. Body temperature will be measured by rectal probe and maintained at 37°C by a circulating water heating pad and heat lamp. Arterial blood gases and pH were monitored with a blood gas analyzer. They were kept within

normal limits by adjusting the volume and/or the ventilation rate, enriching the inspired O2 supply and administration of 8% bicarbonate.

For the cardiac reflex studies in cats, a sinoaortic barodenervation and cervical vagotomy will be performed on both sides of the neck. A midline incision will be made on the front side of the neck. Muscle and connective tissues will be dissected and separated from the common carotid artery. The left and right cervical vagus nerves will be isolated from adjacent the common carotid artery and transected (vagotomy). The carotid sinus nerve is isolated from the internal carotid artery and transected to achieve carotid sinus denervation. The aortic depressor nerve is also isolated from the common carotid artery and transected to achieve aortic barodenervation. The sinoaortic barodenervation is accomplished in order to prevent secondary buffering of cardiac-cardiovascular reflexes by arterial baroreceptors. Vagotomy is performed to allow full manifestation of the excitatory cardiac-cardiovascular reflexes during ischemia and/or chemical stimuli. Successful barodenervation is confirmed by absence of a normal decrease of heart rate in response to an increase in arterial blood pressure induced by administration of phenylephrine and nitroglycerine. This surgery will take about two to three hours.

For cardiac afferent recordings, an incision is made over the sternum located on the midline of the chest. The muscles and connective tissue are removed from over the sternum, which is then cut (sternotomy). The first through the seventh left ribs will be tied off and removed (thoracotomy) to allow access to the sympathetic chain. The left lungs will be ligated around the left pulmonary vein and artery, and left primary bronchus and surgically removed (pneumonectomy). After assessment of anesthesia levels, paralytic agent is then administered prior to nerve bundle dissection. The sympathetic chain will be isolated, placed on a plexiglass platform and covered with warm mineral oil. Small nerve filaments will be dissected gently from the chain between T2 and T5 (medullary cone) with use of an operating microscope. A single nerve fiber is isolated and placed across a recording electrode. At this point, pericardium will be cut to access a coronary artery, and under a surgical microscope, a ligature will be placed around the coronary artery. The ligature will be tightened to cause the coronary artery occlusion, temporarily inducing ischemia. Epicardial application of each of the experimental agents will also be conducted easily after cut pericardium. It will take approximately three to four hours to finish the surgical preparation.

For identification of cardiac sensory neurons in the up thoracic DRGs in anatomic studies in cats, terminal surgery will take place following a 10 weeks recovery period. Anesthesia, cannulation and intubation will be conducted as described in the above first paragraph. After establishing that the cat is physiologically stable, the animal will be deeply

anesthetized with an pentobarbital (150 - 200 mg/kg, iv). An incision is made across the left chest over the 5th and 6th ribs. The ribs are separated and held apart with an abdominal retractor to expose view of the heart. The pericardium is cut open to allow access to the surface of the heart. This surgery will take about one hour. Transcardial perfusion will be performed through the left ventricle using one liter of 0.9% saline solution followed by one liter of 4% paraformaldehyde in 0.5% glutaraldehyde solution. Adequate perfusion is assessed by the stiffness of the head and legs. This will signal the endpoint for this portion of the survival protocol. After cardiac perfusion, the thoracic portion of the spinal cord and dorsal root ganglia will be removed for staining.

For intrapericardial administration of each of the experimental agents in the cardiac reflex studies in rats, a sternotomy is not required. Instead, a small incision is made across the left chest over the 5th or 6th rib. The ribs will be separated and held apart by a retractor to expose the heart. The pericardium will be pinched and a tiny incision is made to allow insertion of a small catheter. The pericardium will be sewn cinched around the catheter. The catheter is used to inject and withdraw chemicals that will be applied to the surface of the heart. After withdrawing the chemicals, saline will be used to flush and wash off excess chemicals on the surface of the heart in the same manner.

Administration of paralytic agent:

After surgical preparations to expose the necessary areas, animals are allowed one hour to stabilize. Animals are assessed for adequate depth of anesthesia as mentioned above. Paralytic agent (gallamine triethiodide, 4 mg/kg) and anesthesia is administered prior to recording cardiac spinal sensory nerve activity and renal sympathetic nerve activity.

Methods to Prevent Dehydration & Hypothermia

Body temperature will be monitored with a rectal probe and maintained at 37°C with a circulating water heating pad or heat lamp. Intravenous drip of 0.9% saline at a rate of 10 ml/kg/hr will be provided as needed according to blood pressure during terminal surgical procedure. If systolic blood pressure falls below 80 mmHg, dextran (6%; 1-2 ml, i.v.) will be administered.

Paralytic Agents

Experimental procedures involve cardiac stimulation. Paralytic agents are required to inhibit spontaneous muscle activity and to reduce muscular reflex contraction due to the stimulation of the heart.

After assessment of appropriate depth of anesthesia, paralytic agent is administered intravenously once after onset of cardiac stimulation. The paralytic agent lasts about 1-2 hrs.

Anesthesia Regimen:

While the animal is under the influence of the paralytic agent, stability of blood pressure and heart rate will be used to assess depth of anesthesia. Arterial blood pressure is monitored through a blood pressure transducer. Heart rate is derived from arterial pressure pulse. Blood pressure may be slightly increased or decreased but not more than 10 mmHg when compared to baseline but remain relatively stable. The heart rate should also be stable. If level of sedation is evaluated to be light, supplemental anesthesia will be administered (See section 7 in the main protocol for dosages). We routinely assess the depth of anesthesia every 15 min and additional doses of alpha chloralose (10-20 mg/kg) will be administered as needed to ensure the adequate depth of anesthesia. In addition, prior to delivery of the paralytic agent we give an additional dose of the anesthesia if needed (dose recommended by the veterinarian in UCI). Assessment of depth of anesthesia before paralytic agent will include lack of reflex to corneal probing and toe pinch, stability of heart rate, blood pressure and body temperature.

Monitoring of adequate anesthesia while paralytic agent is in effect:

While the animal is under the influence of the paralytic agent, stability of blood pressure and heart rate will be used to assess depth of anesthesia. Blood pressure may be slightly increased or decreased but not more than 10 mmHg when compared to baseline but remain stable. The heart rate should remain unchanged. Anesthesia assessments will be compared to the parameters before paralytic agent was administered. If level of sedation is evaluated to be light, supplemental anesthesia will be administered (See section 7 in the main protocol for dosages). We routinely assess the depth of anesthesia every 15 min and additional doses of alpha chloralose (10-20 mg/kg) will be administered as needed to ensure the adequate depth of anesthesia. In addition, prior to delivery of the paralytic agent we give an additional dose of the anesthesia if needed (dose recommended by the veterinarian in UCI). Animals should stabilize and be deeply anesthetized after 2-3 mins after supplemental dosage. The paralytic agent should last approximately 1-2 hrs.

Mechanical ventilation while paralytic agent is in effect:

Animals will be intubated and connected to an artificial respirator. Cats will be ventilated at rate of 200-250 ml/kg/min at tidal volumes of 10ml/kg. Rats will be ventilated at rate of 80-90 breaths/min at tidal volume of 6 ml/kg.

Cat

Rat

Total number of animals

Species	Max
Cat	292
Rat	180

USDA Pain Category	Species	Number of Animals	Number of Animals
USDA Category D	Cat	292	0
USDA Category D	Rat	180	0

Animal Numbers Justification

Explain how the animal numbers were determined.
Uncheck this box to remove all text from the box below

We anticipate that the overall success rate in this investigation will be approximately 65-75% since we may fail to obtain data mainly due to risk of myocardial ischemia, difficulty and complexity of experimental procedures and non-response of the animal to experimental interventions. For instance, animal potentially die from ventricular fibrillation occurred during occlusion of coronary artery. Based on our previous successes in this long-term study and published information we expect close to a 70% success rate for cardiac sympathetic afferent activity studies, a 65-75% success rate for cardiovascular reflexes studies, and a 95% success rate for anatomical studies. Approximately 12 successful animals are needed in each group for generation of statistically significant data. Thus, we are requesting 292 cats and 180 rats for this project based on the following calculations for the three studies:

Cardiac afferent studies, 12 cats / 70% rate → 17 cats/group; cardiovascular reflex studies, 12 cats / 70% rate → 17 cats/group, 12 rats / 65% = 18 rats/group; and anatomic studies, 3 cats / 95% rate → 3 cats/group.

Will animals experience unrelieved pain/distress (category E procedures)?

Drugs & Agents

Anesthesia

Will animals receive anesthesia agents?
Yes
How will the anesthesia be administered to the animals?
Gas
Indicate the anesthesia gas that will be used:
Isoflurane
Administration Method for Gas Anesthesia:
Waste Gas Capture Methods:
Injectable

Species *(receiving the drug/agent)*	Drug/Agent	Dose Range *(mg/kg body wt)*	Route *(SQ, IP, IV, IM, etc.)*	Supplemental Dose (if applicable) *(mg/kg body wt)*
Rat, Cat	Ketamine	30 mg/kg	SQ	
Rat, Cat	*Alpha-chloralose	50-60 mg/kg	IV	10-20 mg/kg
Rat, Cat	Gallamine triethiodide	4 mg/kg	IM, As needed	

Cat	Midazolam	0.2 mg/kg	IM, As needed	

*Justification for Alpha-chloralose:

Recently, many studies [redacted] have employed α-chloralose as anesthetic for experimental animals, which have demonstrated that α-chloralose is a commonly employed anesthetic at many institutions throughout the US and the world in general. For detailed information, please see below referenced publications.

As [redacted] have shown, we can document consistent sympathoexcitatory cardiovascular reflex response by using alpha-chlorolose as one of anesthetic (see references below). Conversely, other anesthetics such as barbital, urethane, and chloral hydrate can depress the sympathetic and cardovascular system and thus could affect the study results ([redacted]

References:

- [redacted]

-

Analgesia

Will animals receive analgesics, sedatives, or other therapeutic agents (e.g. antibiotics, supplemental fluids, etc.)?
Yes

Species (receiving the drug/agent)	*Drug/Agent*	*Dose Range (mg/kg body wt)*	*Route (SQ, IP, IV, IM, etc.)*	*Frequency (how often?)*	*Duration (how long?)*
Rat, Cat	6% Dextran	1-2 ml/kg	IV	As needed	1-3 hours
Rat, Cat	0.9% Saline	0.2-0.4 ml/min	IV	As needed	1-2 hours
Rat, Cat	8% Bicarbonate	0.5 ml/kg	IV	As needed	2-4 hours
Cat	Ampicillin	8 mg/kg	SQ	Procedural	10-12 hours
Cat	Buprenorphine	5-10 µg/kg	IM	Procedural	10-12 hours
Rat, Cat	Lidocaine	3-4 mg/kg	IV	Once	20-30 min
Cat	Bupivacaine	2 mg/kg	SQ	Procedural	6- 8 hours

Experimental & Other Agents

Will animals receive experimental or any other agents (not captured in tables above)?
Yes

Species (receiving the drug/agent)	Drug/Agent	Dose Range (mg/kg body wt)	Route (SQ, IP, IV, IM, etc.)	Frequency (how often?)	Duration (for how long?)
Rat, Cat	Saline	0.9%	Other	Procedural	2 – 5 min
Rat, Cat	Bradykinin	0.5-1 µg/kg	Other	Procedural	5 – 10 min
Rat, Cat	Phenylephrine	10 µg/kg	IV	Procedural	20- 30 min
Rat, Cat	α,β-meATP	1.6 – 6.2 mM	Other	Procedural	5 – 10 min

Rat, Cat	ATP	2 µmol	Other	Procedural	5 – 10 min
Rat, Cat		125-500 µg rat 125-250 µg cat	Other	Procedural	5 – 10 min
Rat, Cat	Nitroglycerine	30 µg/kg	Other	Procedural	10 – 30 min
Rat, Cat	2% Procaine	0.05 – 0.1 mg	Other	Procedural	10 – 20 min
Cat		0.1-1 mg	Other	Procedural	5 – 10 min
Cat		0.2 – 0.4 µmol	Other	Procedural	5 – 10 min
Cat		0.5 – 1 µmol	Other	Procedural	5 – 10 min
Cat		0.2 – 0.4 µmol	Other	Procedural, once	2 – 3 hours
Cat		0.2 – 0.4 µmol	Other	Procedural, once	2 – 3 hours
Cat		1.7 mg	Other	Procedural, once	10 – 20 days
Cat		80 - 160 µg	Other	Procedural, once	2 – 3 hours
Cat		50 µg	Other	Procedural, once	2 – 3 hours
Rat	ADP	25 - 100 mM	Other	Procedural	5 – 10 min
Rat	Histamine	1 - 10 mM	Other	Procedural	5 – 10 min
Rat	TNP-ATP	50-120 µmol/kg	Other	Procedural, once	2 – 3 hours
Rat	RO-3	50-120 µmol/kg	Other	Procedural, once	2 – 3 hours
Rat		5-10 mM	Other	Procedural	30 – 40 min
Rat		5-10 mM	Other	Procedural, once	1 - 2 hours
Rat		5-10 mM	Other	Procedural	30 – 40 min
Rat		5-10 mM	Other	Procedural, once	1 - 2 hours
Rat		5-10 mM	Other	Procedural, once	1 - 2 hours

Will Controlled Substances be used?

Yes

Indicate which controlled substances will be used - Check all that apply:

Buprenorphine

Ketamine

Sodium Pentobarbital (aka. Euthasol, Nembutal, Fatal Plus)

CSUA #:

Uncheck this box to remove all text from the box below

Will non-pharmaceutical grade (i.e. chemical grade) agents be used in live animals?

No

Animal Locations & Husbandry

Food or Water Variations

Indicate the food or water variations that will be implemented in this protocol - Check all that apply:

Justification for the Food or Water Variations

Animal Husbandry Variations

Indicate the animal husbandry variations that will occur - Check all that apply:

Justification for the Animal Husbandry Variations

Researcher-Maintained Animals

Will LAB STAFF provide basic routine husbandry and care for the animals?

Will animals be held in the lab (outside of the vivarium) for more than 12 hours?

An Emergency Plan is REQUIRED for the following scenarios:

Other Husbandry/Housing Variations

Describe any other variations or special considerations (not already captured in the sections above).

Animal Locations

Indicate all locations where live animal procedures and/or housing will take place - Check all that apply:
ULAR Vivarium
Lab areas (outside of the ULAR vivarium) OR non-ULAR vivarium

Building	Room #	Location will be used for:		
		Non-surgical Procedures	Surgeries	Housing > 12 hrs
			X	
			X	

Will any live animals (owned by UCI) be taken to offsite (non-UCI) locations for procedures?
No

Hazards & Safety

Chemical Hazards

Requirements for the Use of Potentially Hazardous Chemicals or Agents:

Biological Materials, Primary Cells or Cell Lines

Requirements for Use of Biological Materials:

Infectious Agents

Requirements for the Use of Infectious Agents:

Recombinant DNA

Preparation & Use of rDNA

Radioactive Hazards

Radiation Use Authorization (RUA) #:

Removal of Radioactive Waste & Monitoring of Radioactivity

Creation of New Transgenic Animals

Are you creating a NEW strain of transgenic animal by crossbreeding 2 different strains?

Animal Biosafety Levels

Indicate the animal biosafety levels - Check all that apply:
ABSL-1

Other Hazards or Safety Considerations

Other Protocol Information

Provide any other information about this animal-use protocol (that is not captured in any other sections or tabs).

Personnel

Principal Investigator (PI)

Faculty Sponsor

Co-Investigator/Senior Researcher

Research Personnel

Other Research Personnel

Additional Personnel Information

Emergency Contact Information
Uncheck this box to remove all text from the box below

Name	Phone #	
	Daytime	After-hours *(mobile/cell preferred)*

Add New User

Training Requirements

PI Qualifications

Description of PI Qualifications
Uncheck this box to remove all text from the box below

has performed research for years using cats, dogs, pigs, rabbits, mice and rats at and UC Irvine. is the principal investigator of this project and will conceive all protocols and review or write all manuscripts resulting from the proposed studies. will be responsible for directing all experimental procedures in this project and conduct some experiments described in the protocols.

Training Plan for Study Team Members
Uncheck this box to remove all text from the box below

	has gained experience at

	has worked with feline and rodents as a post-doctoral graduate researcher for years at and as a faculty researcher at UC Irvine for years. has also performed survival surgery on rats at will be responsible for conducting some experiments described in the protocols.
	has had years of research experience on rats and cats from , as well as post-doctoral research experience at and UC Irvine . has also performed survival surgery in cats and rats at UC Irvine. will also be responsible for performing all experimental protocols.
	A laboratory technician will assist with surgical preparations in anesthesia, blood-gas analysis, and artificial ventilation for terminal procedures.

Undergraduate students who have been trained by staff and faculty researchers will assist with surgical preparations in anesthesia, blood-gas analysis, and artificial ventilation for terminal procedures. They will be trained annually according to additional requirements from IACUC. They will take part during terminal experimentation, blood gas analyses and cardiac perfusion.

Links to Other Protocols

Other Regulatory Review Requirements

Indicate if other regulatory reviews are required for this protocol - Check all that apply:

If additional details need to be discussed regarding any of the links above, describe them below.

Number	Protocol Title	Document Template
		Animal Use Protocol (AUP)

PI Certification

PI Certification

I hereby acknowledge and assure the following:
By clicking this checkbox, I certify that the above statements are understood and will be followed by all research team members.