UCI Office of Research
Research Management System

Animal Use Protocol

Title

General Information

Project Overview

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

Cardiovascular illness is a major health concern in today's society and a leading cause of death in the United States. The body reacts to cardiovascular dangers such as during cardiac angina (chest pain) or myocardial ischemia (decreased blood flow to an artery) with symptoms of increased heart rate and increase in blood pressure throughout the body. A huge portion of the population has high blood pressure, in particular, in the middle-aged and older population. High blood pressure leads to heart problems and strokes (bleeding in the brain). Although there are many medications to decrease blood pressure, a large portion of the patients does not have their blood pressure under control. As such, we continue to investigate ways to treat or minimize hypertension. The findings will help human and animals with high blood pressure to seek integrative medical therapy such as acupuncture to lower the blood pressure. The increase in sympathetic activity participates in these ailments. On the other hand, too low blood pressure and heart rate lead to fainting or syncope. These are generalized cardiovascular responses. There is evidence that low frequency electroacupuncture can be used to treat and have therapeutic effects on increases and decreases in blood pressure and heart rate and sympathetic activity. However, the mechanism for which electroacupuncture works is not well understood. Our laboratory is actively investigating mechanisms in several regions (listed below) of the central nervous system (CNS) responsible for the regulatory inhibition of electro-acupuncture on cardiovascular responses such as pressor (increased blood pressure) and depressor (decreased blood pressure). By defining the mechanism of electroacupuncture, we can provide scientific evidence for the use of electroacupuncture as an alternative or integrative treatment for various cardiovascular conditions. The benefits of acupuncture are: almost no side effects, less costly, could work in conjunction with conventional pharmaceutical treatment.

Study Characteristics

Surgery

Types of Surgery

Terminal (animals will not recover from anesthesia)

Minor

Major

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 examining the responses of the brain nuclei to electroacupuncture (EA). thus, paralytic agents must be used to prevent flexor movement and activation of non-EA-induced input to the brain.

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

Overall, that somatosensory nerve stimulation with electroacupucnture and thermal stimulation with moxibustion involve the brain regions to modulate sympathoexcitatory responses and elevated sympathetic tone. Sympathoexcitatory responses include increases in central cardiovascular neuronal activity and peripheral sympathetic nerve activity, arterial blood pressure, heart rate

and tachyarrhythmias. that parasympatho-excitation can be modulated with electroacupuncture. Moreover, cardiac sensory nerve stimulation evokes increases in blood pressure and involves specific brain region.

The articles generated from these findings are listed below:

1

2

3

4

5

Justification for Continuing the Project

Uncheck this box to remove all text from the box below

that a number of regions in the brain such as rVLM, Arcuate nucleus, PVN that are important in the regulation of blood pressure, we will continue to explore other cardiovascular regions that are important in the regulation of high blood pressure. We will examine the involvement of acupuncture in these regions. We will explore regions in the pons such as the parabrachial nucleus.

It is important to know exactly the brain regions involved in the actions of acupuncture in addition to blood pressure regulation. In other words, the somatosensory nerves (for instance located in the arms and legs) activated during acupuncture modify the cardiovascular regions of the brain to reduce the abnormal activity during high blood pressure. Previously, we have examined cardiovascular regions in normal conditions. We would like to continue to study the regions that are affected by high blood pressure as well and can be influenced by acupuncture therapy to reduce blood pressure.

Funding Source	Funding Status	Award/Proposal #	Billing Account #
	Awarded		

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

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

Based on scientific literature and previous studies, rats and cats are the most appropriate species for this proposed study. The cardiovascular effects of acupuncture are very similar in rats and cats. Cats are used to determine the functional projection related to acupuncture from one region to another in the brain.

The rats are used to reduce the number of cats. By studying in rats the acupuncture related projections in how they contribute to the processing of the actions of acupuncture through the neurotransmitter systems.

following reasons also show why cats are one of the best species for the study:

1) Many laboratories around the world use cats to conduct acupuncture-related studies

. This indicates that the cat is frequently used animals for this kind of study. Furthermore, we can easily obtain information from previous studies regarding experimental methods to refine our techniques and procedures to minimize pain, distress, and discomfort in the animals.

2) The brain regions and acupoints in the cat have been well studied and mapped. Therefore, the cat are reliable species for this study. In addition, the cardiovascular effects of acupuncture are very similar in rats and cats . The advantage of cats is their size, which facilitates placement of multiple or closely spaced recording and stimulating electrodes for electrophysiological experiments, which technically is impossible to be performed in the rat.

3) has established excellent cat models to obtain consistent pressor (increase in blood pressure and heart rate) responses induced by gastric distension and activation nerve endings on the gallbladder. Studies using the cat models have been published in several well-known scientific journals.

4) has established and published on phenylbiguanide (PBG) induced decreases in blood pressure and heart rate responses in cat. Studies on central processing concomitant with multiple central pathways and circuitry of this inhibitory reflex will be conducted in cats.

5) No evidence has yet shown that other small species of animals can be used for this study. In respect to this, neither guinea pigs nor swine serve as adequate models to study cardiovascular responses caused by stimulation of abdominal organs as we normally perform in our laboratory. In addition, a swine cannot be used as an animal model because the acupuncture points and the specific brain regions in swine have not yet been clearly defined.

References

Animal Characteristics

List the specific strains that will be used.
Uncheck this box to remove all text from the box below

Normal healthy cats 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

Vertebrate animal model is necessary to study reflex cardiovascular responses to abdominal stimulation. The pathway for these reflex responses involves the central nervous system. Multiple systems will be involved in this study. In addition, the effect of acupuncture on blood pressure can only be evaluated in living animals. Thus, non-animal models cannot be used for this study.

Based on scientific literature and previous studies, the rat also is another appropriate species for this proposed study. The cardiovascular effects of acupuncture are very similar in rats and cats (The following reasons also show why the rat is the best species for the study:

1) Many laboratories around the world use rats to conduct acupuncture-related studies . This indicates that the rat is frequently used animal for this kind of study. Furthermore, we can easily obtain information from previous studies regarding experimental methods to refine our techniques and procedures to minimize pain, distress, and discomfort in the animals.

2) The brain regions and acupoints in the rat have been well studied and mapped. Therefore, the rat is a reliable species for this study.

3) has established an excellent rat model to obtain consistent pressor (increase in blood pressure) responses induced by gastric distension. Studies using this rat model have been published in several well-known scientific journals. It suggests that this rat model is an appropriate model for the proposed study. In addition, not changing the animal model will save additional animals that would be needed to establish a new experimental model.

4) No evidence has yet shown that other small species of animals can be used for this study. In this respect, neither guinea pigs nor swine serves as adequate models to study cardiovascular responses caused by stimulation of abdominal organs as we normally perform in our laboratory. In addition, a swine cannot be used as an animal model because the acupuncture points and the specific brain regions in swine have not yet been clearly defined.

References

Animal Characteristics

List the specific strains that will be used.
Uncheck this box to remove all text from the box below

Spontaneous hypertensive rat, the Dahl salt-sensitive rat, and Spraque Dawley rat with normal blood pressure 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
Jul-30-2019	Jan-01-1975	Jul-30-2019	PubMed	9	acupuncture, sympathoexcitatory, cardiovascular responses, rat
Jul-30-2019	Jan-01-1975	Jul-30-2019	PubMed	0	acupuncture, sympathoexcitatory, cardiovascular responses, cat
Jul-30-2019	Jan-01-1975	Jul-30-2019	Web of Science	23	acupuncture, sympathoexcitatory, cardiovascular responses

Database Searches

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

Previous research shows that various studies have been performed involving acupuncture and various conditions like inflammatory pain, spinal pain, and epilepsy have been treated. However, these previous acupuncture studies looked at different acupuncture effects such as gastric motility and arthritis. Thus, our study is not an unnecessary duplicate since the projected goal of our study is to investigate cardiovascular responses to visceral stimulation and electroacupuncture.

Furthermore, literature and [redacted] have demonstrated that the reported brain regions are specific regions in the brain that are important in the regulation of blood pressure. However, there are no other studies indicating the effect of acupuncture on the cannabinoid system and the estradiol system in these brain regions. We will study these regions by investigating how the cannabinoid system as well as the estradiol system involved in processing acupuncture-generated stimulation inputs and effects.

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

A vertebrate animal model is necessary to study reflex cardiovascular responses to the stimulus of cardiac, abdominal or cardiopulmonary afferent stimulation. The pathway for these reflex responses involves the central nervous system and thus non-animal models will not suffice. Multiple systems will be involved in this study. In addition, the effect of acupuncture on blood pressure and heart rate can only be evaluated in living animals. Fully functional cardiovascular and nervous systems need to be intact to observe the full effects and mechanisms of acupuncture on the cardiovascular responses. The pathway for these reflex responses involves the peripheral and central nervous system and thus non-animal models will not suffice. Thus, non-animal models cannot be used for this study

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 since has published the most extensive literature available on the topic of the cardiovascular responses following acupuncture. The few studies available suggest some alternative species that would not be appropriate for the proposed studies. We encourage the reviewer to read a few of in rats and cats. to study the action of acupuncture on cardiovascular function. We now have published well over studies and always strive to reduce the number of animals.

The effectiveness of acupuncture is around 75% in both humans and animals. Thus, the number of animals necessary to study the mechanisms reflects this effectiveness. We are in the process to examine how to increase the effectiveness of acupuncture. In order to reduce the number of animals used, more than one protocol is performed on each animal. The intention is to reuse the rat that has been examined for saline control. These control rats will be good subjects for the test protocols such as microinjection of an antagonist. Thus, the rats will undergo more than one experimental procedure. Sometimes we can record from more than one neuron enhancing the sample size of recorded cells and therefore reduce the number of animals. The animal's physiological state is stabilized after each experimental procedure in order to ensure that further studies are capable of being performed and, more importantly, that the animal is anesthetized and comfortable. In addition, the treatment of acupuncture relaxes and reduces pain in animals.

We are performing more experiments in rats than cats to study the beneficial effects of electroacupuncture in decreasing the high blood pressure. As such, we are reducing the number of cats.

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

Previous studies have been conducted on areas of the brain that have been included in this study, but there no studies investigating the roles of these areas involving the mechanisms of acupuncture. One study found investigates the effect of acupuncture on gastric signal vagal efferent fibers but only suggests that acupuncture may adjust by either increasing or decreasing intragastric pressure. Another study examines the neuronal activity in the nucleus tractus solitarius with ear acupuncture but did not determine how the acupuncture effect influences hypertension. We are looking at the cardiovascular responses resulting from the actions of acupuncture so thus our study is not a duplicate of any other previous work.

No related alternatives have been found to replace paralytic use to prevent muscular activity interfering with acupuncture stimulation of the median or sympathetic nerve. All of the non-verbal behavioral and physiological signs used for recognizing pain in humans can be sought for in animals, at least in those of comparable construction to humans. While similarity of nervous systems to that of humans is an important criterion used in judging whether or not an animal might feel pain, specifying design criteria does not specify the solution. Taken together, the similarities with humans suggest that many other animals have similar subjective experiences to humans and similar assessment for responses to pain.

We are constantly refining our experimental procedures to limit the surgical time and the extensiveness of the surgery. 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. We encourage the veterinary staff and any member of IACUC to come visit our laboratory to view our procedures.

Study Segments

Experimental Design	Species
actions acupuncture	Rat,Cat

actions acupuncture

Species

Cat
Rat

Experimental Design Summary

Does this study segment tab describe the establishment and maintenance of a breeding colony?
No - This study segment tab describes EXPERIMENTS (complete all questions below)

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: Hypertension is multifactorial and includes over excitation of the sympathetic nervous system such as during a sympathoexcitatory cardiovascular reflex response and the condition hypertension. Hypertension is prevalent and causes morbidity and mortality. The proposed studies will continue to study the cells and brain regions responsible for this disease and how stimulation of somatosensory nerves with acupuncture can reduce the excitation of the neurons and hence blood pressure. By examining the sympathetic and parasympathetic systems responsible for increases and decreases in blood pressures, respectively, we can understand better how to improve acupuncture therapy in blood pressure control. Finally, the neurotransmitter systems that are suggested to influence blood pressure are proposed as potential mechanisms in the central actions of acupuncture.

The abbreviations used are listed below

- rVLM – rostral ventrolateral medulla
- cVLM – caudal ventrolateral medulla
- IML - intermediolateral column of the spinal cord
- NA – nucleus ambiguus
- NTS – nucleus tractus solitarii or nucleus of the solitary tract
- PVN – paraventricular nucleus
- PBN - parabrachial nucleus
- PBG – phenylbiguanide
- Kyn - kynurenic acid
- KA - kainic acid
- DLH - DL-homocysteic acid
- E2 – 17 beta-estradiol
- FAAH – fatty acid amide hydrolase inhibitor URB597
- AM251 – CB1 (cannabinoid receptor 1) antagonist
- CCK – cholecystokinin
- GABA – gamma aminobutyric acid

-

Rationale for the examination of different regions in the brain on blood pressure regulation and control of blood pressure: The goal of this study is to identify the mechanisms of inhibition by the central nervous system (CNS) during electroacupuncture (EA). We have identified the areas of the brain involved with EA such as the rostral ventral lateral medulla (rVLM), arcuate nucleus (ARC), and nucleus ambiguus (NA). Our studies have led us to investigate the neurochemical modulators involved during EA and various pathways of inhibition by EA.

Our current protocols will involve studying the roles and types of chemical neurotransmitters in the brain and spinal cord not studied before. We will also be classifying and recording the cellular activity of cells in various brain regions (rVLM, cVLM, NA, NTS, PVN) that are involved in sympathetic and parasympathetic nervous systems to improve the acupuncture therapy by inducing sympathoinhibition and parasympathoexcitation. We also will study a new region in the pons, that could be involved in actions of acupuncture. Thus, we will evaluate the mechanisms underlying the central processing of the actions of electroacupuncture on sympathoexcitation during elevated blood pressure, and sympathoinhibition and parasympathoexcitation during cardiopulmonary reflex responses including decreases in blood pressure and heart rate.

The studies include approaches:

1. Identifying a neuron communicating from one brain region to another (antidromic stimulation), recording of neuronal activity.

2. Delivery of agonist and antagonist in minute amounts onto the cell being recorded (iontophoresis) to examine how the brain regulate blood pressure and how acupuncture gets processed during elevated blood pressure.

3. Characterization of neurons for cardiovascular responses to increase of blood pressure by using phenylephrine and decrease of blood pressure using nitroprusside.

4. Microinjection to study role of specific neurotransmitters in the brain with EA and without EA.

5. Pressor reflex model, which will induce an increase in blood pressure and heart rate through either electrical stimulation of the splanchnic nerve or chemical stimulation, by application of bradykinin to the gallbladder or gastric distention; each response is separated by at least 10 min.

6. Depressor model mimics conditions of decreased blood pressure and heart rate by stimulation of the cervical vagus nerve or intravenous injection of PBG; each response is separated by at least 10 min.

Typical routine of the approaches (1, 2, 3) used to study the cell: In order to trace the pathways or study the neuronal projections from one cardiovascular region to another, we will test for collision of spikes. This technique involves collision of antidromic spikes generated with antidromic stimulation and orthodromic spikes (spontaneous spikes and orthodromic stimulation such as convergent input). Investigating these areas involve experimental microinjection and administration of chemicals to classify the types of cells and their responses to agonists and antagonists with and without acupuncture. For instance, stimulating rVLM will send information up to the cell body located in the PVN. Then the cell body will be studied for its involvement in endocannabinoid or estradiol E2 during the actions of acupuncture and absence of acupuncture. The neuron also is examined for its characteristic. Since we study blood pressure regulation, we need to test the cell for its response to increase of blood pressure by using phenylephrine and decreased of blood pressure using nitroprusside.

We will be using the following test groups: (please refer to Drugs & Agents for list of chemicals).

Pressor and sympathoexcitatory responses (15 groups of rats, each group =12 rats)

Part 1.

Cardiovascular responses: Role of the PVN or and rVLM in EA on pressor and sympathoexcitatory responses through endocannabinoid system in rats.

Investigating these areas involve experimental microinjection and administration of chemicals to examine their pressor responses and sympathoexcitatory responses (Approach 5) to agonists and antagonists (Approach 4) with and without acupuncture.

- Repeated pressor reflexes to evaluate time control and location. (1 group)
  a. To locate site of microinjection and visceral input: KA or Kyn into PVN or or rVLM; once per nucleus during 10 repeated pressor responses (n=6).
  b. To locate site of microinjection and visceral input: saline into PVN or or rVLM; once per nucleus during 10 repeated pressor responses (n=6).
- Repeated pressor responses (10 every 10 min) before and after EA. (2 groups)
  a. To study the role of with KA/Kyn during EA effect; microinjection after EA application (n=6).
  b. To study if DLH excitation of cells in mimic EA effect (n=6).
  c. To study the communication between rVLM with KA and with DLH without EA and then with EA (n=6).
  d. To study the communication between PVN with KA and with DLH without EA and then with EA (n=6).
- (4 groups)
  a. (n=12).
  b. (n=12).
  c. (n=12).
  d. (n=12).

Part 2.

Neuronal mechanism: Role of the PVN or and rVLM in EA on pressor and sympathoexcitatory responses through endocannabinoid system in rats. (8 groups, each group =12 rats)

Investigating these areas involve experimental microinjection and administration of chemicals to classify the types of cells and their responses to agonists and antagonists with and without acupuncture. (Approaches 2, 3)

- Neuronal recording of splanchnic nerve evoked activity in PVN or or rVLM. (2 groups)

a. Recording of cells in PVN and then (n=12).

b. Recording of cells in and then rVLM (n=12).

- Neuronal recording in PVN or or rVLM with EA and saline control iontophoresis. (2 groups)
  a. Recording of cells in rVLM and then saline control iontophoresis in (n=12).
  b. Recording of cells in rVLM and then saline control iontophoresis in PVN (n=12).
- (2 groups)
  a. (n=12).
  b. (n=12).
- (2 groups)
  a. (n=12).
  b. (n=12).

Part 3.

(9 groups, each group = 12 rats)

Investigating these areas involve experimental microinjection and administration of chemicals

- (4 groups)
  a. (n=12).
  b. (n=12).
  c. (n=12).
  d. (n=12).

(4 groups)

a. (n=12).

b. (n=12).

c. (n=12).

d. (n=12).

- (n=6) (n=6)

Part 4.

Classification of Cell Types and neuronal pathways in cats (6 groups, each group = 8 cats)

In order to trace the pathways or study the neuronal projections from one cardiovascular region to another, we will test for collision of spikes. This technique involves collision of antidromic spikes generated with antidromic stimulation and orthodromic spikes (spontaneous spikes and orthodromic stimulation such as convergent input). We will also induce increases and decreases in blood pressure and heart rate by intravenous administration of nitroprusside (decrease) and phenylephrine (increase) to categorize the neuron that is sensitive to EA. (Approach 1, 2, 3)

- rVLM antidromic stimulation and PVN recording. Then PVN recording of EA sensitive neuron Administration of IV phenylephrine or nitroprusside to test for cardiovascular responses of the neuron. (Cat, n=8)

- . (Cat, n=8)

- rVLM antidromic stimulation and PVN recording. Then PVN recording of EA sensitive neuron Administration of IV phenylephrine or nitroprusside to test for cardiovascular responses of the neuron. (Cat, n=8)

- [redacted] (Cat, n=8)
- [redacted] (Cat, n=8)
- [redacted] (Cat, n=8)

Depressor and parasympathoexcitatory responses

Part 1

Role of the [redacted] and NTS and cVLM and rVLM in EA on parasympathoexcitatory cardiopulmonary responses through opioids system. (6 groups, each group =12 rats)

Investigating these areas involve experimental microinjection and administration of chemicals to examine their depressor responses to agonists and antagonists with and without acupuncture.

- Repeated depressor reflexes to evaluate time control and location. (2 groups)
  a. To locate site of microinjection and visceral input: KA or Kyn into [redacted] or NTS or cVLM or rVLM; once per nucleus during 10 repeated pressor responses (n=12).
  b. To locate site of microinjection and visceral input: saline into [redacted] or NTS or cVLM or rVLM; once per nucleus during 10 repeated pressor responses (n=6) and with EA (n=6).
- Repeated depressor responses (10 every 10 min) before and after EA: role for [redacted] or NTS or cVLM or rVLM. (4 groups)
  a. To study the role of opioids with ICI or CTOP blockers during EA effect; microinjection in [redacted] (n=12) [redacted] NTS after EA application (n=12).
  b. To study the role of opioids with ICI or CTOP blockers during EA effect; microinjection in cVLM (n=12) or rVLM after EA application (n=12).

Part 2.

Neuronal activity: Role of the and NTS and cVLM and rVLM in EA on parasympathoexcitatory responses through opioids system. (11 groups, each group =12 rats)

Investigating these areas involve experimental microinjection and administration of chemicals to classify the types of cells and their responses to agonists and antagonists (iontophoresis) with and without acupuncture.

- Record vagal evoked neuronal activity in NTS, cVLM, rVLM or NA with EA and opioid agonist, antagonist or saline control iontophoresis (Approaches 2 and 6). Then, each cell will be recorded for cardiovascular and neuronal responses to phenylephrine and nitroprusside (Approach 3).
  a. (n=12).
  b (n=12).
  c. Recording of cells in NTS with EA and then iontophoresis of opioid agonist or saline control (n=12).
  d. Recording of cells in cVLM with EA and then iontophoresis of opioid antagonist (n=12).
  e. Recording of cells in cVLM with EA and then iontophoresis of opioid agonist or saline control (n=12).
  f. Recording of cells in rVLM with EA and then iontophoresis of opioid agonist or saline control (n=12).
  g. Recording of cells in rVLM with EA and then iontophoresis of opioid antagonist (n=12).
  h. Recording of cells in NA with EA and then iontophoresis of opioid agonist or saline control (n=12).
  i. Recording of cells in NA with EA and then iontophoresis of opioid antagonist (n=12).
  j. Without acupuncture iontophoresis of opioid agonist in NTS, cVLM, rVLM or NA (n=12).
  k. Without acupuncture iontophoresis of opioid antagonist in NTS, cVLM, rVLM or NA (n=12).

Part 3.

(3 groups of rats)

Investigating this area involves experimental microinjection and administration of chemical:

- (n=12).
- (n=12).
- (n=12)

Animal Monitoring

Animal Monitoring Details

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

Multiple surgical sites may result in loss of bodily fluids. Loss of blood will prevent oxygen from flowing to the body, causing hypercapnia. In result, high carbon dioxide and water will induce acidosis. This can be seen through blood gas. Pain /distress is non-applicable since depth of anesthesia is monitored every 15 min. (see below in section Bb for monitoring)

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

Monitoring parameters that will be used to assess pain, distress and discomfort in animals:	A slight drop in body temperature (1-2°C) is expected due to animal being under anesthesia. An increase or decrease in blood pressure and heart rate is expected (cardiovascular responses) during experimental procedures (afferent stimulation and injection of chemicals). 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 palpebral reflex and muscle movement or trembling will also indicate that the animal is 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.
Management plan that will be used to assess and treat pain, distress and discomfort in the animals, including any special procedures that will be used (e.g., periodic weighing of animals):	Blood pressure and heart rate is constantly monitored throughout the experiment with special attention to any signs that animal is coming out of anesthesia (Please see section 5.B for sedation assessment). In addition, blood gas analyses are checked periodically to maintain animal homeostasis. Supplemental anesthesia (please see section 7 for dosages) is given as needed.
Frequency in which laboratory staff will be monitoring the animals, in addition to the daily general observations made by ULAR vivarium staff:	Blood pressure, heart rate and sedation levels constantly are monitored throughout the experiment. In addition, blood gas analyses are checked periodically (every 15 minute) to maintain animal homeostasis. Supplemental anesthesia (please see section 7 for dosages) is given as needed.

Documentation of Animal Monitoring

Lab notebook

In animal room (vivarium)

Endpoints

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

At the end of each study, dye is microinjected to aid in identifying probe tip location, which signals the experimental endpoints. Animals will be kept under anesthesia for ~9 hours. After the final collection of data, animals are given pentobarbital (200 mg/kg, iv) or 1 M of KCL (1 ml/kg, iv) for euthanasia.

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

Every attempt is made to keep animals in stable physiological condition. In the case that an individual animal suffers surgical trauma and cannot recover to stabilized condition, the animal may be removed from the study ahead of schedule. Such conditions may include cardiac arrest, severe blood loss, and fluid accumulation in lungs leading to respiratory failure, brain tissue damage due to trauma or deprivation of oxygen (hypoxia) or blood flow (ischemia) or extreme acidosis. Treatments may include defibrillations, intravenous drips of ringer's solutions, thoracentesis (to drain pleural fluid) or administration of therapeutic drugs. Animals will be removed from study and euthanized if treatments do not remedy the situation and health of animal continues to decline.

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	KCl	1 ml/kg of 1 M KCL	IV
cat	KCl	1 ml/kg of 1 M KCL	IV
rat and cat	Pentobarbitol	100-200 (mg/kg body wt)	IV

Confirmation of Death in Animals

Other

Brain tissue removal.

Other

Termination of heart rate and blood pressure for over 5 minutes.

You MUST...

Animal number calculation for experimental part actions acupuncture

Cat		
	Max	Description
	140	
	140	**Cat**

Rat		
	Max	Description
	1400	
	1400	**Rat**

Copy IACUC SOP templates

Procedure Descriptions

Terminal Procedures

Euthanasia followed by Tissue Harvest: Cats and rats are euthanized by anesthetic overdose of KCl or Pentobarbital. Brain tissue will be harvested for histological confirmation of the sites of microinjections and recordings.

Surgical Procedures

Pre-operative Care

Routine husbandry and housing provided by ULAR prior to surgery

Induction of Anesthesia for Surgical Procedures in Live Animals

Indicate how anesthesia/sedation is induced for non-surgical procedures: *Cat* - Initial anesthesia is induced by subcutaneous injection of Ketamine Hydrochloride. Anesthesia (alpha-chloralose) is given intravenously into the brachial vein prior to cannulation procedures with butterfly catheter. *Rat* - Initial anesthesia is induced by subcutaneous injection of ketamine and xylazine. Thereafter anesthesia (alpha-chloralose) is given intravenously femoral or jugular. Supplemental anesthesia (alpha-chloralose) is also given intravenously in both cats and rats.

Describe how the level of anesthesia/sedation is assessed to be adequate to begin the non-surgical procedure: 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 palpebral reflex test or noxious toe pinch and muscle movement or trembling will also indicate that the animal is coming out of anesthesia. In addition, inconsistency in respiration with the ventilator also indicates animal is not fully anesthetized.

Describe how animals are monitored throughout the non-surgical procedure: Animals are monitored for stable blood pressure and heart rate and absence of response to noxious stimulation (paw pinch or corneal probing) to determine level of depth of anesthesia every 15 min. 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). A rectal probe is inserted to monitor the animal's body temperature that is maintained with a heating pad and/or heat lamp. Supplemental anesthesia is given as needed according to details described above for level of sedation. Once proper depth of anesthesia is established, blood pressure and heart rate will be monitored for stability. The toe pinching, palpebral reflex test, and respiration also ensure the animal is well anesthetized. Regular breathing pattern is monitored at all times. In addition to administration of regular additional doses of alpha-chloralose to maintain depth of anesthesia, palpebral reflex test is evaluated every hour. Before paraplegic agent is provided, necessity of alpha-chloralose, absence of fluctuation in blood pressure and absence of any corneal reflex are evaluated. Hourly additional dosages of alpha-chloralose are administered to ensure maintenance of sedation throughout the study.

If animals will be placed on artificial ventilation, describe the range of respiration rates and tidal volumes: Rats will be ventilated at rates of 80-90 breaths/min at tidal volumes of 6 ml/kg. Cats will be ventilated at rate of 200-250 ml/kg/min at tidal volumes of 10ml/kg.

Aseptic Techniques

Preparation of the surgical space: A clean surgical table is designated for surgery. Prepare with a water-heating pad to keep the animal warm, sterile absorbable paper for a clean platform and contain blood material, and drapes to ensure airborne sterility.

Preparation of the surgeon: Clean surgical scrubs and gloves will be worn.

Preparation of the animal: All surgical sites will be shaved and cleaned with sterile saline.

Sterilization of instruments: All surgical instruments will be sterilized by treatment in an autoclave

Methods to Prevent Dehydration & Hypothermia

Body temperature will be monitored with a rectal probe and maintained at 36°C with a circulating water heating pad and/or heat lamp. If systolic blood pressure falls below 80 mmHg, 6% dextran (1-2 ml, iv) and 0.9% saline (0.2-0.4 ml/min, iv drip) will be administered to maintain blood pressure above 80 mmHg.

Post-Operative Care and Analgesic Usage

N/A - Terminal Surgery Only

Terminal Surgery (Description of Procedures)

Initial anesthesia is induced by subcutaneous injection of Ketamine Hydrochloride and xylazine. Anesthesia will be administered intravenously with butterfly catheter into the brachial vein. After administration of alpha-chloralose, cannulation will take place. 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. The cat will be intubated with a cuffed endotracheal tube. Intubation of the rat will be performed through a tracheotomy (incision in the throat to access trachea or windpipe) to allow insertion of an uncuffed endotracheal tube. Body temperature will be measured by rectal probe and maintained at 99.5 - 102.5 F (37.5 - 39.1 C) by a circulating water heating pad and heat lamp. Arterial blood gases and pH will be monitored with a blood gas analyzer. The subjects' blood gases will be kept within normal limits by adjusting the volume and/or the ventilation rate, enriching the inspired O2 supply and administration of 8% bicarbonate.

Stimulation of gallbladder or stomach:

- A 1-2 inch incision will be made flank just below the last right rib. Tissue will be dissected to expose the gallbladder beneath. The site is kept open to allow placement of pledgets soaked in BK.
- Gastric distension: a small balloon will be inserted down the esophagus into the stomach. About 3-5 ml of air will be injected to inflate balloon causing gastric distension resulting in a pressor or depressor response.
- For (electrical) stimulation by splanchnic nerve: The splanchnic nerve will be isolated through a 1-2 inch flank incision on the side of the body from the bottom of the last rib to just above the location of the adrenal gland and kidney. An electrode is attached for stimulation.
- For splanchnic nerve input neural recording, posterior, inferior of the skull will be removed in order to position an electrode into the nucleus ambiguus, nucleus tractus solitaries, rostral or caudal ventrolateral medulla or paraventricular nucleus. Cells will be recorded in these locations.

Stimulation of vagal afferents

Other femoral vein will be cannulated located on the upper thigh. Catheter will be measured and inserted up to the inferior vena cava to the entrance of the right atrium. Thereafter 3-5 CC of phenylbiguanide (100mM) will be intravenously injected to induce a cardiovascular reflex.

For (electrical) stimulation by cervical vagus nerve: The vagus nerve will be isolated through a 1-2 inch flank incision on the side of the neck. An electrode is attached for stimulation.

Microinjections

Animals will be placed in a stereotaxic head frame with steel plugs introduced through the auditory meatus and bars holding the upper jaw to fix the head rigidly. Craniotomy will be performed to expose the surface of the brain. The hole for exposure will be 2mm in circular radius for a dorsal approach for microinjection, stimulation and recording extracellular activity in the cVLM, rVLM, NA, NTS. A single modified microdialysis probe or a multi-barrel glass micropipette will be positioned at the dorsal surface of the brain to reach the brain regions. The locations will be identified preliminarily by the pressor and tachycardia responses during chemical stimulation and confirmed histologically.

Neural recording

The animals will be placed in a stereotaxic head frame with steel introduced through the auditory meatus and bars holding the upper jaw to fix the head rigidly. Craniotomy will be performed to expose the dorsal surface of the brain. The recording glass pipette will be inserted using the specific coordinates according to the Berman atlas. The electrodes fabricated with the Sutter electrode puller are high impedance electrodes. Once a desired neuron is identified, the recording procedure will last for one to two hours. We will be able to record one neuron per experiment per animal in experiments that examine the characteristic response to EA. At times we will be able to record more than one cell in control experiments, such as saline microinjection or influence of drug on the reflex. In other experiments, we will microinject blockers or stimulate in one area to record the activity of the neuron located in another nucleus

All protocol procedures that will involve a craniotomy to access areas of the brain:

After the animals' head is placed on stereotaxic apparatus, a midline incision is made along the top of the head and excess tissue is removed to expose the skull. Anatomical landmarks such as bregma (the junction point of the sagittal and coronal sutures on top of the skull) and lambda (junction where cranial sagittal suture and lambdoid sutures at the back of the skull) and are located used for location of brain regions. Burr holes (about 3 - 5 mm) are drilled through the skull overlying regions of the hypothalamus ( ). The occipital bone is removed to expose the medulla for access to regions of the rVLM, cVLM, NA, and NTS.

Probe insertion:

A single modified microdialysis probe or a multi-barrel glass micropipette will be positioned at the dorsal surface of the following brain sections.

The locations will be identified preliminarily by the pressor and tachycardia responses during chemical stimulation and confirmed histologically. The sites of microinjection and iontophoresis will be marked by spots of Chicago sky blue injected in.

For studies involving the IML (spinal cord):

An incision (about 3 -5 inches) will be made over the thoracic region of the spine (T1-T8). Excess muscles and tissues will be removed to expose the thoracic vertebras. The removal of vertebras T2 or T3 will allow exposure of spinal cord and access to IML. The dura mater surrounding the brain and spinal cord will be carefully removed to allow insertion of probes or electrodes of corresponding protocol.

Administration of paralytic agent:

After surgical preparations to expose the necessary areas, animals are allowed 1 hour to stabilize. Animals are assessed for adequate depth of anesthesia. Paralytic agent and anesthesia is administered prior to cellular recording.

Renal sympathetic nerve activity (RSNA) recording:

Following a flank incision, an electrode will be wrapped around the renal nerve to record RSNA. Integrated rectified multiunit will be measured by subtracting baseline discharge activity determined by crushing nerve after the experiment.

Microdialysis:

Two microdialysis probes (CMA 20) will be implanted into the rVLM. Artificial cerebral spinal fluid will be perfused at 2 µl/ml through a pump and dialysate from the probe collected continuously for 5 min with a refrigerated fraction collector.

USE OF PARALYTIC AGENTS (NEUROMUSCULAR BLOCKING AGENTS)

Indicate which procedure(s) described in the Experimental Design require the use of paralytic agents and why: Experimental procedures involve EA treatment. Paralytic agents are required to inhibit

spontaneous muscle activity and to reduce muscular reflex contraction due to EA stimulation.

How the paralytic agent will be administered, including the point in the surgical/experimental procedure at which it is first administered and approximately how long animals will be under its influence: After assessment of appropriate depth of anesthesia, paralytic agent is administered intravenously once after onset of EA. The paralytic agent lasts about 1-2 hrs.

Describe the anesthesia regimen that will be used while the animals are under the influence of the paralytic agent. Indicate how the depth of anesthesia will be assessed prior to administration of the paralytic agent: 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. 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 palpebral reflex and response to toe pinch, stability of heart rate, blood pressure and body temperature.

How will animals be monitored for adequate depth of anesthesia while the paralytic agent is in effect (are supplemental doses of anesthesia needed?): 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. 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. Lastly and most importantly, to tell if the changes in blood pressure and hare heart rate are due to changes in depth of anesthesia or due to the treatments, we assess the palpebral reflex of the animal. If there is no palpebral reflex, which indicates the hemodynamic changes are due to the treatments. Otherwise, it indicates inadequate depth of the anesthesia.

In addition to the administration of regular additional doses of alpha-chloralose to maintain the depth of anesthesia, pressor responses to noxious foot pinch are evaluated every hour to monitor the depth of anesthesia after administration of the paralytic agent. Because the previous study indicates that after paralysis, adequate depth of anesthesia can be determined by lack of pressor responses to noxious foot pinch

How mechanical ventilation will be performed while the paralytic agent is in effect, e.g. equipment used, details of tidal volume and respiration rate: 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 rates of 80-90 breaths/min at tidal volumes of 6 ml/kg.

Guidelines for Paralytic Use in Live Animals:

1. A surgical plane of anesthesia must be established and verified prior to administration of the paralytic agent; this anesthesia level must be maintained during the entire time that the agent is in effect.
2. Endotracheal intubation and provision for mechanical ventilation must be initiated prior to the administration of the paralytic agent.
3. Use of paralytic/neuromuscular blocking agents should be confined solely to the phase of the procedure for which they are indicated.
4. During the period of paralysis, multiple physiologic indicators of pain and distress (e.g., heart rate, blood pressure) must be monitored at least every 15 minutes as appropriate to the species and recorded in the surgical record. An increase of >20% in any monitored parameter should be considered indicative of a pain/stress response and additional doses of anesthetic must be administered. The use of automated monitoring devices, however, cannot substitute for direct monitoring of the animal by a human observer. A member of the laboratory staff must be present at all times while paralytic agents are in use.
5. The use of end-tidal carbon dioxide monitoring is strongly recommended to ensure adequate ventilation.
6. Core temperature and fluid balance must be maintained within normal levels during the period of paralysis. In the event that animals will be under the influence of the paralytic for long periods of time (e.g., more than 4-6 hours), a urinary catheter must be placed or the urinary bladder must be manually voided.
7. Animals must be spontaneously breathing before anesthesia is discontinued. Research personnel must confirm that animals have fully recovered control of respiration and locomotion at the end of the experiment, before they are returned to their home cages.

Rat

Cat

Total number of animals

Species	Max
Cat	140
Rat	1400

USDA Pain Category	Species	Number of Animals	Number of Animals
USDA Category D	Cat	140	0
USDA Category D	Rat	1400	0

Animal Numbers Justification

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

Cats:

Our current success rate for studies involving antidromic stimulation is approximately 50% in cats. The success rate of 50% associated with the antidromic procedure is determined by identifying a cardiovascular neuron that projects to another cardiovascular nucleus. To achieve significance a minimum of 8 data points are needed per group. Therefore, 6 groups X 8 data points = 48 data points and 48 cats / 0.5 = 96 cats needed

The success rate of about 50% is typical for the technique antidromic stimulation. This method allows us to find and determine the characteristic of the neuron. This means that we will know if the neuron that responds to acupuncture and projects as a single cell from one brain region to another one participates in the neuronal network important for the actions of acupuncture therapy. This level of complexity and identification of the specific neuron yield a success rate of 50%.

Rats:

We strive to execute multiple protocols in one subject and thus 52 groups of rats needed.

Success rate is 80%, which is related to the success of cannulation, craniotomy and repeatable reflex responses. A total of 12 rats are needed to acquire significance. Therefore, 52 groups X 12 = 624 and at 80% rate we need 624/0.8 = 780 rats.

Will animals experience unrelieved pain/distress (category E procedures)?
No

Drugs & Agents

Anesthesia

Will animals receive anesthesia agents?
Yes

How will the anesthesia be administered to the animals?
Injectable

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	KCl	Saturated (4g/ml/kg)	IV	Once (euthanasia)	N/A
Rat, Cat	Pentobarbital	150-200	IV	Once (euthanasia)	N/A
Cat	Ketamine	30	SC	Once	During procedure
Rat	Ketamine	75-100	SC	Once	During procedure
Rat, Cat	Alpha-chloralose	50-60	IV	Once	During procedure
Rat, Cat	Alpha-chloralose	5 mg/kg/hr	IV	continuously	2-4 hours
Rat, Cat	Gallamine triethiodide	4	IV	As needed	2-4 hours
Rat	Xylazine	5-10	SC	Once	During procedure

*Justification of using Alpha-chloralose as anesthetics for experimental animals:

Recently, many studies 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 check the following list of publications.

Reference List:

-
-
-
-
-
-
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Some of these studies utilize Urethane in conjunction with alpha-chlorolose; we prefer to not use urethane in conjunction with alpha-chloralose since we propose to investigate the synaptic transmission of neurotransmitters. We are concerned that urethane will interfere with our investigation. Urethane interferes with neurophysiological studies as shown in the article presented by the link

Additionally, previous study has shown a combination of anesthetics namely ketamine and alpha-chloralose. The article presented by the link describes the use of ketamine/xylazine with alpha-chloralose.

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

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?)*
Cat	Bradykinin	1-10 ug/ml (Vehicle Used: 0.9% saline; Total Volume: 1ml)	Other	As needed	1-2 min
Rat, Cat	Potassium chloride	1 M (Vehicle Used: 0.9% saline; Total Volume: 1ml/kg)	IV	Once	1-5 min
Rat, Cat	Gabazine	27 mM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	Once	1-2 hours
Rat, Cat	Naloxone	1 mM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	Once	1-2 hours
Rat, Cat	Saline	0.9% (Vehicle Used: water; Total Volume: 50 nl-0.1 ml)	Other	As needed	1-5 min
Rat, Cat	Nitroglycerin	30 µg/kg (Vehicle Used: water; Total Volume: 0.5-1 ml)	IV	Once during procedure	30 min
Rat, Cat	Phenylephrine	10 µg/kg (Vehicle Used: water; Total Volume: 0.5-1 ml)	IV	Once during procedure	30 min
Rat, Cat	Kainic Acid (KA)	1 mM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	Once during procedure	30 min
Rat, Cat	Kyurenic acid (Kyn)	1 mM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	Once during procedure	30 min
Rat, Cat	D,L-homocysteic	4-8 NM (Vehicle Used: 0.9%	Other	Once	0.5-1 hour

	acid (DLH)	saline; Total Volume: 100nl)		during procedure	
Rat, Cat	Phenylbiguanide (PBG)	40 mg/kg (Vehicle Used: 0.9% saline; Total Volume: 1ml/kg)	IV	As needed	1-2 min
Rat, Cat	GABA	50 nM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min
Rat, Cat	Naloxone	10 nM (Vehicle Used: 0.9% saline; Total Volume: 50-100nl)	Other	As needed	30 min
Rat, Cat	CTOP	10-20 nM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min
Rat, Cat	ICI-174,864	30 nM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min
Rat, Cat	Nor-BNI	50 nM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min
Rat	Phosphate buffered saline (PBS)	0.1 mM (Vehicle Used: water; Total Volume: 20 µl)	Other	Once during procedure	1-5 min
Rat	dimethylsulfoxide (DMSO)	5% (Vehicle Used: water; Total Volume: 20 µl)	Other	Once during procedure	1-5 min
Rat, Cat	DADLE	10 NM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min
Rat, Cat	Losartan	10 mM (Vehicle Used: 0.9% saline; Total Volume: 50nl)	Other	As needed	30 min

Will Controlled Substances be used?

Yes

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

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 over years using cats, dogs, pigs, rabbits, and rats at and UC Irvine. will conduct some experiments described in the protocols and supervise all aspects of the protocol.

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

Name	Qualifications, Level of Training, and Research Responsibilities
	as 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.
	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 the will be responsible for conducting the experiments described in the protocols.

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.