[redacted]

Title: **Tibia fracture model with plate fixation**
Sponsor Name: **SUNDRY**
PI Name: [redacted] Protocol #: [redacted] Type: **Current View**
Species: **RATS** # Of Animals: **32** Date Received: **April 04, 2019**

Study Staff

Name	Role	Degree	Organization
[redacted]	Other		[redacted] > [redacted]
[redacted]	Research Technician		[redacted]
[redacted]	Research Technician		[redacted] > [redacted]
[redacted]	Principal Investigator	Ph.D	[redacted] > [redacted]
[redacted]	Consultant	MS	[redacted] > [redacted] > [redacted]
[redacted]	Research Technician		[redacted] > [redacted]

Non Study Staff

Name	Degree	Organization
[redacted]	MD	[redacted] > [redacted]

Linked Agreements

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]	[redacted]	[redacted]	[redacted]	SUNDRY	RM – Research Sundry	IR	[redacted]	Approved

Protocol Overview

Please answer the following questions using language a non-scientist will understand.

1. Study Goals

How would you explain the long term or overall scientific goals of the proposed work to a non-scientist? **[Please limit to 200 words.]**

In this pilot study, we will create a tibial fracture in a rat model and repair the fracture with a plate and screws. The purpose of this study is to look at the feasibility of capturing the recovery of the animals by gait analysis, static weight bearing, and reflex response testing. We plan to use this model and endpoint measurements to ultimately compare the efficacy of locally administered non-opioid analgesics. Our long-term objectives focus on

[redacted]

demonstrating (1) that local post-operative non-opioid analgesia can be as effective as systemic opioid analgesia in addressing post-surgical pain in a tibial fracture model with plate fixation and (2) that local post-operative non-opioid analgesics with antibiotic properties can effectively treat postoperative infections. These are future goals - the proposed pilot study does not use locally delivered non-opioid analgesia.

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This field may contain information that has been migrated from **Insight 3.6.4, Detailed Research Plan, section A. Goals**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Study Goals** question above. ***Use of this information is optional.***

2. Benefit to be Gained by Animal Research

How would you explain to a non-scientist that the potential benefits of the study, in terms of biomedical advancement, justify the proposed animal use? **[Please limit to 200 words.]**

Tibial shaft fractures occur at an incidence of 16.9/100,000/year [[redacted] et al, Injury, 46, 4 (2015)]. Most often these patients are prescribed opioids for long periods of time making the patients susceptible to opioid addiction. Due to the widespread detriments of systemic opioids, there is a current need to develop multi-modal analgesia regimens to treat post-surgical pain including non-opioid medication. Our approach aims to address this need. Currently, there is no reliable local delivery system for addressing post-surgical pain. The long-term objective of this pre-clinical work is to develop a local delivery gel to apply to the plate used in tibial fracture fixation that elutes out pain medication.

In addition, a costly complication of internal fixation of tibia fracture is infection, which occurs in about 11% of patients [redacted] et al, Injury, 47 7 (2016)]. In order to deal with such healthcare burden, it is necessary to clarify the pathological mechanism and develop effective therapies. We hypothesize that using non-opioid analgesic-eluting gel applied to the plate during tibial fixation will allow us treat acute infection in tibial fractures.

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This field may contain information that has been migrated from **Insight 3.6.4, Detailed Research Plan, section B. Background**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Benefit to be Gained** question above. ***Use of this information is optional.***

Research Objective: Research Objective 1

[redacted]

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [Please limit to 200 words]

The long-term objective is demonstrating that local post-operative non-opioid analgesia via a controlled release coating on fracture plates can be as effective as systemic opioid analgesia in addressing post-surgical pain in a tibia fracture fixation model. All patients undergoing tibia fracture suffer from varying degrees of post-operative pain. Based on the recovery of tibia fracture patients, there is a need to address pain by medication for up to 8 weeks. This study aims to develop the surgical method by which a fracture can be created and fixed using a plate which can be performed in a clinically relevant manner for tibial metaphysis fractures. In this pilot study, we expect to determine the timeline of bony healing of the fracture by histology, micro-CT (ex vivo) and correlating it to functional outcomes using gait metrics. The method determined as a result of this study will be used in further studies assessing the efficacy of local pain medication release from coatings on fracture plates. The central hypothesis for our long-term studies is that the use of systemic opioids can be decreased by local delivery of non-opioid pain medication.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Overall research plan:

All rats will undergo a metaphyseal transverse osteotomy of the proximal tibia to simulate a tibial fracture. Fracture fixation will be performed using a titanium Y-plate and titanium alloy screws. We will monitor the rats' gait over time by video analysis and nociceptive pain sensitivity measurements (von Frey). Below are the study groups (n=8 each, n=32 total).

Pilot group (32 animals): Tibial fracture and plate fixation

- Sacrificed at 2 weeks for uCT imaging (n=8)

- Sacrificed at 4 weeks for uCT imaging (n=8)

- Sacrificed at 6 weeks for uCT imaging (n=8)

- Sacrificed at 8 weeks for uCT imaging (n=8)

Time course: up to 61 days (4 days preconditioning time for measurements; 56 days post-surgically)

Potential side effects: Related to surgical procedures - infection or death during anesthesia or in the peri-operative period. Opioids can also depress respiration rate.

Experimental endpoints: We do not expect or plan on peri-surgical infection. Thus, sign of infection such as skin necrosis and pus will be endpoints for euthanasia. We will also look for early signs of infection such as swelling and will check the animal's temperature if we suspect infection. Functionally, some lameness or changes in gait are expected during the study. Non-weight bearing lameness or complete off-loading of the limb in stance or in gait is expected commonly in the first two weeks after surgery, but this type of lameness for more than 14 days is indicative of an unexpected outcome and will invalidate our measurements of functional pain. Therefore, this is a criteria for terminating the study. 15% weight loss is also a criteria for euthanasia as it is indicative of the general well being of the animals being significantly compromised.

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This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 1. Study Duration**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

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This field may contain information that has been migrated from **Insight 3.6.4, Tumor Production, question 4. "What are the experimental endpoints used for this tumor study?"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

[redacted]

1. Describe the health status of the animals during this research objective. Include:

   - Expected development and progression of clinical signs, including severity and time course
   - Potential adverse events caused by the research model and/or experimental manipulations
   - If a scoring system will be used to monitor animal health, please attach it to the protocol below.

The goal of this study is to quantify the differences in the pain response and 'functional pain' of the animals and the time course of these responses in a tibia fracture model. In general, the animals are not expected to be compromised by the surgical procedure. No diseases, tumors, toxic or paralytic agents will be applied / introduced. The procedure should not restrict the rats from moving freely around their cage. The animals may be expected to unload or partially load their surgical limb for the first two weeks post-surgically. After this period, the animals are expected to be loading their surgical limbs. We will monitor general well being by observation and weight measurements.

Rats will be monitored twice daily during the first 3 days, and then daily. Investigators will inspect the incision for any signs of gross infection, edema, exudate, non-healing or wound dehiscence. Any sutures/staples will be removed 7-10 days after surgery. In addition, the animals' ability to load their surgical limb and the time course of functional recovery will be monitored visually, by allowing them to walk in the gait arena with video recording (see procedure), toe spread measurements from the videos (see attached abstract) and nociceptive responses using von Frey measurements.

The procedure involves creating a metaphyseal tibia fracture and inserting a plate and screws to stabilize said fracture. This is a major injury as well as a major surgery which is expected to produce pain both during surgery as well as for a period of time after surgery. We will be administering a pre-determined regimen of buprenorphine pre-operatively and for 72 hours post-operatively which simulates what is done clinically at the hospital after plating of the tibia. However, we have classified the animals into Category E because after this period, they may still be experiencing some pain which will not be alleviated by pain medication.

Pain is expected because of the nature of the procedure, but we expect no other health problems.

2. What action will be taken should clinical signs manifest?

Pre-operative: Buprenorphine 0.05 mg/kg SC or IP single dose

Post-operative: Buprenorphine 0.05 mg/kg SC or IP every 6-12 hrs (standard schedule will be every 8 hours) for 72 hrs

[redacted]

Rats will be placed on a warming pad (circulated water) and observed until they fully recover from the isoflurane anesthesia. Post-operatively, animals will be observed twice a day during the first 72 hours. After 72 hours, rats will be observed once a day.

Because we eventually plan to investigate the effects non-opioid analgesics local versus systemic opioids, we do not intend to provide additional analgesics including NSAIDs other than those planned for each group. We will monitor the animals for unexpected signs of distress such as reduced grooming, porphyrin staining, reduced level of spontaneous activity, piloerection, hunched posture, squint-eyes, increased aggressiveness when handled, or distance themselves from cage mates, reduced food/water intake.

The ultimate goal in the long term (not the pilot) is to determine the comparative effectiveness of locally delivered pain medication and that of systemically delivered opioids. Since we are looking to determine quantitatively the differences in the listed endpoint measurements and are looking to determine the progression of post-surgical injury/recovery and pain separately, we have a pre-determined schedule of medication delivery and will not administer any other medication to address pain. Since this is a pilot study, the exact time course of recovery is not yet known. We will work with our facility veterinary staff to limit the animals' discomfort by alternative methods such as diet supplementation.

We do not expect to see gross or systemic signs of infection such as skin necrosis, pus or centrally elevated temperature. These would be criteria for termination.

Functionally, some lameness or changes in gait are expected during the study. Non-weight bearing lameness or complete off-loading of the limb in stance or in gait is expected commonly in the first two weeks after surgery, but this type of lameness for more than 14 days is indicative of an unexpected outcome and will invalidate our measurements of functional pain. Therefore, this is a criteria for terminating the study. 15% weight loss is a criterion for euthanasia as it is indicative of the general well being of the animals being significantly compromised. We will be monitoring the weight of the animals every other day and if we observe sudden weight loss, we will work with our facility veterinary staff to recover their weight by diet supplementation.

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This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 2, "Describe the investigator's responsibilities during the post-surgical and/or post-experimental period..."**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

[redacted]

Attachments

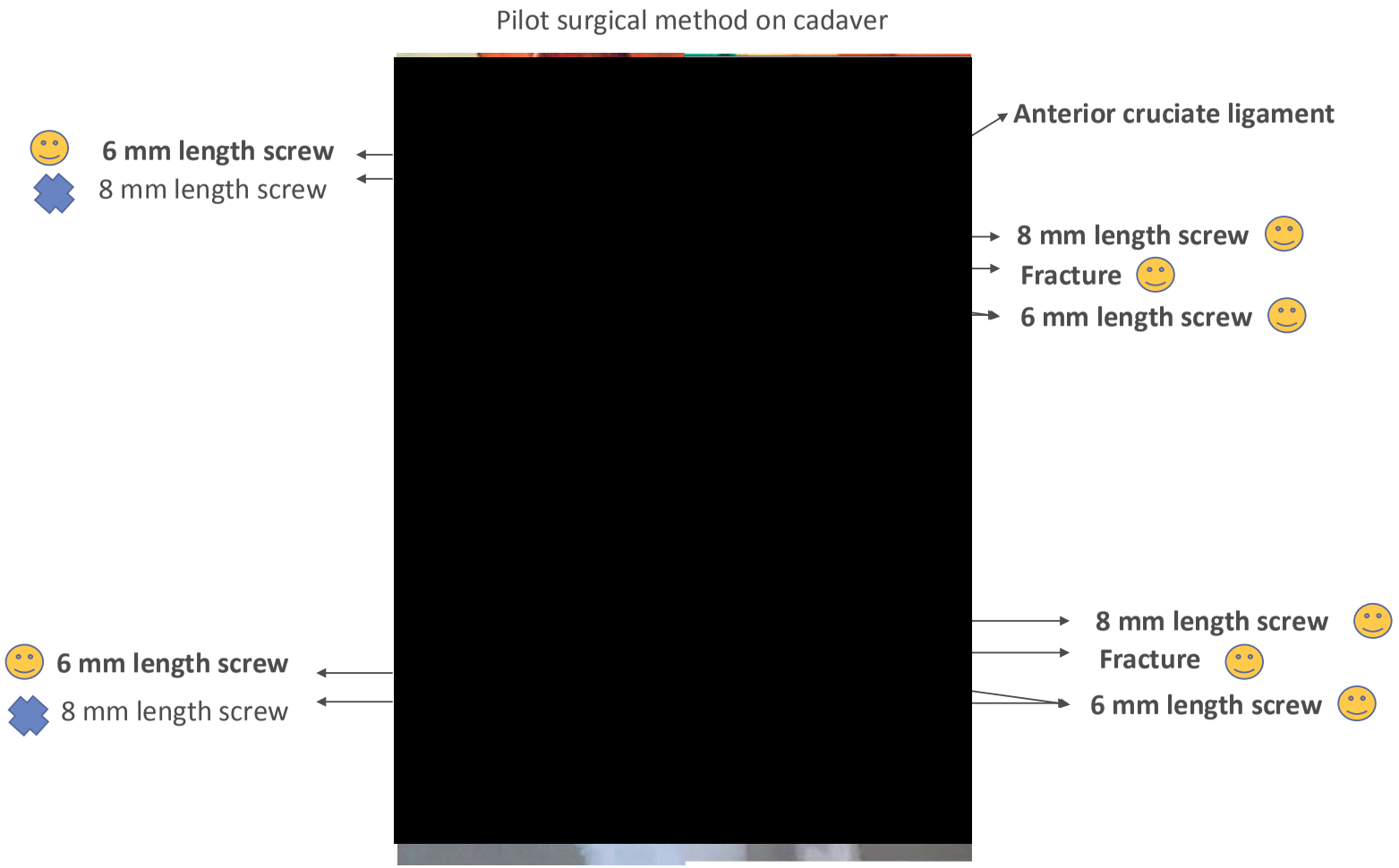
Name	Mode
Flowchart 20190207 (Flowchart)	Electronic

Tibial fracture and plate fixation - rats

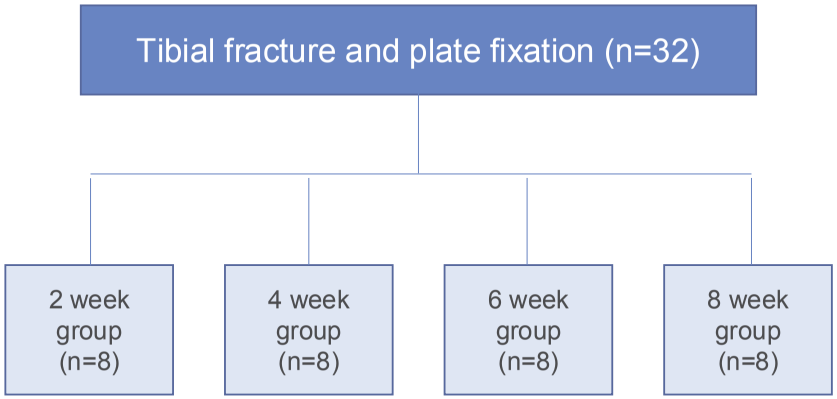
Pilot Study

February 2019

Surgery


Pilot surgical method on cadaver
6 mm length screw
8 mm length screw
Anterior cruciate ligament
8 mm length screw
Fracture
6 mm length screw
6 mm length screw
8 mm length screw
8 mm length screw
Fracture
6 mm length screw

Study Groups

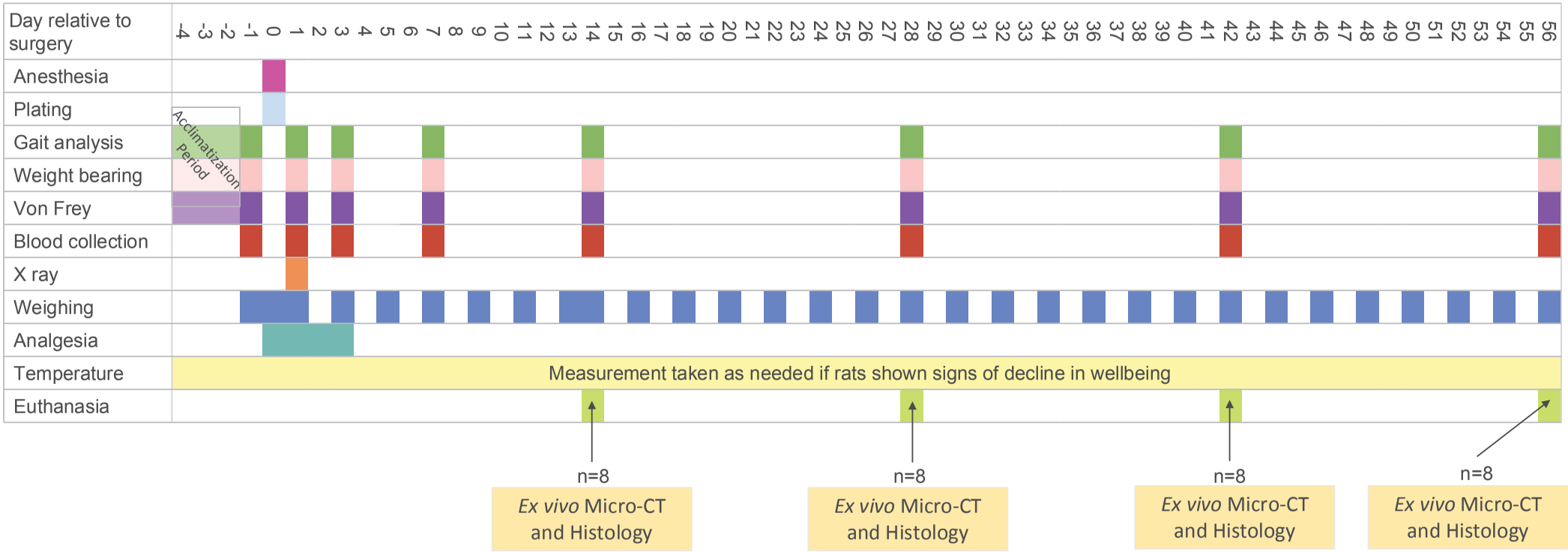


Study Outline

	Male Sprague Dawley Rats (n=32)			
Pre-op analgesia	Preemptive buprenorphine 0.05 mg/kg SC or IP			
Procedure	Tibial cutting with saw, plate fixation over injured bone with screws			
Intro-op anesthesia	Isoflurane 1-3% inhalant to effect (up to 5% for induction)			
Plate specifications	Titanium “Y” shaped plates (1.8 cm length; 0.7 mm thick)			
Screw specifications	8 mm x 1.1 mm; 6 mm x 1.1 mm self-drilling screw (titanium alloy – titanium, aluminum, and niobium)			
Post-op analgesia	Postoperative buprenorphine SC or IP for 3 days (up to 0.05 mg/kg)			
In vivo (days)	2 weeks/14 days (n=8)	4 weeks/28 days (n=8)	6 weeks/42 days (n=8)	8 weeks/56 days (n=8)
In vivo endpoints	Weight monitoring: Every other day X-ray, blood collection, weight bearing, pain analysis and gait analysis: POD -1, 1, 3, 7, 14, 28, 42 and 56 [some have a few additional measurements, see slide 4 for details]			

- Rats are sacrificed at 2 week, 4 week, 6 week, and 8 week timepoints so that ex vivo uCT can be performed.
- This is a pilot study to determine if our endpoint pain and gait measurement can be used in a fracture model
- If successful, we will amend the protocol to add a control group and additional treatment groups, but we are not doing that at this time to reduce the number of animals used before we confirm that our post-op measurements are appropriate.

Study Timeline



[redacted]

Animals

The IACUC restricts protocols to a single species only. If the protocol will require xenografts, identify the donor species, and the applicable protocol number, in the appropriate **Research Objective** section of the protocol.

1. Select a species from the drop down list:
Rats (Rattus)

1.a Select breed(s), or strain(s), or specific type(s).

Sprague Dawley

Please list other breed(s), strain(s), or specific type(s). See FAQ for institution-specific examples.

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2. Do any of the animals have a genetic alteration and/or phenotype that is expected to have any impact on animal health and/or requirements for animal care?

○ Yes ◉ No

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3. Animal Source
Select all that apply:

☑ Animals will be acquired from an approved vendor - no quarantine is required. See FAQ for institution-specific approved vendors.
☐ Animals will be acquired through import. See FAQ for institution-specific procedures.
☐ Animals will be bred as part of this protocol
☐ Animals will be transferred from another protocol at this institution
☐ Animals will be acquired from an outside institution

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4. Sex

◉ Male ○ Female ○ Both

If you will be using one sex only, please explain:
Our primary endpoint measurement, gait analysis, is sensitive to the weight of the animals. i.e. it is best to keep variations in weight to a minimum. If we use female animals, we will have to match weight ranges and therefore would be using male and female rats of significantly different ages even in the case when we can have similar weights. Further, previous

[redacted]

studies have suggested that response to pain and pain medications (as well as possibly gait analysis metrics) are expected to be different between the two genders (For example, [redacted]. 2009 Neuroscience 10:283). Therefore, identifying gender differences is not a part of current study and would require using double the number of animals at a minimum to identify. For these reasons, we have chosen to use male rats. We also have an extensive database of healthy males animals (~140) for our gait, von frey, weight bearing, and toe spread measurements and we would have to recreate this database if we were to use female animals.

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5. Indicate the method(s) of identification that will be used to track these animals *(select all that apply)*:

- ☐ Implant/microchip (See FAQ for SOP)
- ☑ Ear tag or notch (See FAQ for SOP)
- ☐ Tattoos (See FAQ for SOP)
- ☐ Collar
- ☑ Cage card
- ☐ Other

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6. The species chosen is appropriate because *(select all that apply)*:

- ☑ The process resembles that in humans
- ☑ Prior research has been conducted in this species
- ☐ Tissues and/or other substances needed are best/uniquely provided be this species
- ☑ Species lower on the phylogenetic scale cannot be used
- ☑ The size or anatomy of this species is best/uniquely suited to the procedure(s)
- ☑ Tissues and/or other substances to be harvested require an animal of this size
- ☐ Other

Potential Pain and Distress

1. Total number of animals requested for this three-year approval

Enter the number of animals in each pain and distress category. Each animal must be assigned to a category based on the most invasive procedure or the procedure that has the greatest potential to cause pain or distress. See FAQ for definitions and examples.

- If an animal will be used in more than one **Research Objective**, count it only once, in the highest pain category that it will experience.
- If animals are bred in-house, include the progeny that may be culled. Progeny used for experiments should be counted in the specific **Research Objectives**. All other animals should be counted in **Other** as follows:

[redacted]

	Category
Breeders	B
Progeny culled without genotyping	B
Progeny culled after genotyping (<21 days old)	C
Progeny culled after genotyping (>21 days old)	D

TOTAL NUMBER OF ANIMALS REQUESTED
USDA Pain & Distress Category (See FAQ for information)

Animals	B	C	D	E	Total
Research Objective 1			0	32	
Other (e.g. breeding, training):					
Total requested	0	0	0	32	32
Animals currently in house					
Total approved for purchase	0	0	0	32	32

2. Justification for the number of animals requested (select all that apply):

☐ Power analyses indicated that the proposed sample size, number of groups and/or number of experiments is the lowest required for statistically valid tests of the hypothesis (i.e., 80% power with 0.05 type I error).
☑ Differences from controls are expected to be small, and large sample sizes are necessary to distinguish differences reliably.
☑ Based on previous and/or published data, the numbers of animals requested are the minimum needed to achieve sufficient statistical power.
☐ These animals will be used to produce antibodies or tissues, and numbers are based on yield.
☐ The numbers of animals or group sizes have been established by federal guidelines/requirements.
☑ This is a pilot/feasibility study that uses the minimum number of animals required to provide meaningful, but not statistically significant data (i.e., model development).
☐ This model involves breeding of genetically modified rodents. Based on Mendelian genetics, it is expected that ¼ of all pups will be homozygous and ¼ will be wild type, with the remaining ½ heterozygous. The homozygous and wild type mice will be used to generate data for the experiment, and the heterozygotes will be used to replace the breeding stock or will be euthanized.
☐ Other

The following tools can be used to determine minimum sample size:

- Sample Size Calculations in Animal Research (W. W. LaMorte, BUMC)
- ClinCalc Sample Size Calculator
- Jackson Laboratories Breeding Colony Size Planning Worksheet

[redacted]

3. Does the number of animals requested include extra animals to cover anticipated failures or to train or familiarize the staff with the procedures described?

O Yes ◉ No

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4. The protocol includes animals in USDA Pain & Distress Category E, animals subjected to potentially painful or stressful procedures that are not relieved with anesthetics, analgesics, and/or other drugs; therefore, strong scientific justification must be provided.

a. Explain the procedure(s) that will produce pain and/or distress.

The procedure involves creating a metaphyseal tibia fracture and inserting a plate and screws to stabilize said fracture. This is a major injury as well as a major surgery which is expected to produce pain both during surgery as well as for a period of time after surgery. We will be administering a pre-determined regimen of buprenorphine pre-operatively and for 72 hours post-operatively. However, we have classified the animals into Category E because after this period, they may still be experiencing some pain which will not be alleviated by pain medication.

b. Provide scientific justification why pain and/or distress cannot be relieved with anesthetics, analgesics, and/or tranquilizers. State the reasons why relief of pain and/or distress would interfere with test results.

This is a pilot study looking at the feasibility of capturing the recovery of animals from this major injury and repair surgery by gait analysis. We plan to use this model and endpoint measurements to ultimately compare the efficacy of locally administered non-opioid analgesics. Therefore, the administration of analgesics has to be strictly controlled and pain medications that are not a part of the study including NSAIDs cannot be administered. We plan on administering opioid medication for 72 hours peri-surgically in this set of animals to simulate the clinical peri-operative period for trauma patients in the hospital.

While typical pain management for fracture fixation includes an NSAID in addition to an opioid, we will not be administering NSAIDs because eventually we will be comparing this set of animals with groups receiving locally delivered non-opioid pain medication from a hydrogel. These will include groups with NSAID-impregnated hydrogels, so administering NSAIDs in the control group we are proposing in this pilot study would interfere with the comparisons we plan to make with treatment groups in the future. We have categorized the animals as E because this is a pilot study and we do not know if they will experience pain after the 72 hour window of buprenorphine administration.

c. Describe any measures that will be used to minimize pain and distress (e.g., special bedding, supplemental food, heat packs, etc.)

We will monitor the animals for clinical signs of pain and distress such as lack of grooming, porphyrin staining, hunched posture, aggressive behavior and will

[redacted]

evaluate the necessity of addressing their health status in co
our facility veterinarians. We will give diet supplementation as needed as an alternative to pain medication.

32

Replacement, Reduction, and Refinement

The 3 Rs – replacement, reduction, and refinement – represent a practical strategy for researchers to apply when considering the use of animals in research and in designing humane animal research studies. Government policy and regulatory agencies require the IACUC to assure that researchers consider the 3 Rs when preparing research protocols.

- The Guide for the Care and Use of Laboratory Animals
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- USDA Policies 11 and 12

1. Alternatives to Animal Models

☑ Mathematical models are not a suitable alternative to live animals
☑ Computer simulations (in silico models) are not a suitable alternative to live animals
☑ In vitro biological systems are not a suitable alternative to live animals
☐ Other

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2. Duplication of Research

Unnecessarily duplicative research should be avoided for scientific and ethical reasons. Have the results fulfilling the experimental goals of this study been published in medical, scientific, or veterinary journals?

O Yes ◉ No

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3. Search for Alternatives to Painful and/or Distressful Procedures

A literature search for alternative procedures must be performed for each procedure that has the potential to cause pain or distress, including prolonged use of restraint devices. Along with the literature search, consultation with experts in the field and attendance at scientific or professional meetings can be used to identify alternatives to painful and/or distressful procedures.

a. Indicate resources used to search for alternatives to painful and/or distressful procedures. In addition to the selections below, other useful resources can be found at IACUC Central , the NIH Office of Laboratory Animal Welfare, and the USDA Animal Welfare Information Center.

☐ Medline (http://library.massgeneral.org/)

[redacted]

☑ Pubmed (http://library.massgeneral.org/)
☐ Agricola (https://agricola.nal.usda.gov/)
☐ ALTBIB (https://toxnet.nlm.nih.gov/altbib.html)
☐ ALTWEB (http://altweb.jhsph.edu/)
☐ Animal Welfare Institute (https://awionline.org/)
☐ Google Scholar (http://scholar.google.com/)
☐ Other databases (please list):
☐ Consultation with experts with knowledge of alternatives within this specific field. Provide name(s) and qualifications/credentials, date, and content of the consultation.
☐ Scientific/professional meetings attended to remain current with pertinent information regarding alternatives in this specific field. Provide meeting name, date, and relevant topic.

b. Indicate the date the literature search was completed. The search must be conducted within the last 6 months.

Click here to enter a date.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Bii, Date of Literature Search**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search Date** field above. Literature search dates for field above for all migrated protocols were defaulted to the date of migration. ***Use of this information is optional.***

c. Indicate the time period surveyed in the literature search:
1979-2019

d. Indicate the procedure(s) and keyword(s) searched for each potentially painful or distressful procedure or condition described in this protocol.

Procedure	Keywords
Procedure 3: Tail bleed	Keywords: blood collection, tail vein, rat, alternatives.
Procedure 1: Tibial bone plate	Keywords: rat surgical pain model, fracture, bone plate, gait analysis
Procedure 2: Euthanasia	Keywords: rat CO2, euthanasia guidelines rat, CO2 flow rate rodent euthanasia; pentobarbital, alternatives

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Biv: "Indicate the procedure and keyword(s) used"**. The information in this section could not be mapped from your approved application to a new

[redacted]

form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search** Date field above. ***Use of this information is optional.***

e. Results of the Search for Alternatives to Painful and/or Distressful Procedures

☑ The literature search conducted indicates that there are no alternative procedures that would involve less pain or distress.
☐ There are alternative procedures, however, they cannot be used for these experiments.

If there are any relevant citations or other documents that are needed to support this search for alternatives, please attach them to this form.

Humane Endpoint Disposition and Euthanasia

A. Humane Endpoints

Mammals:

- Persistent recumbence; inability to rise; loss of righting reflex
- Pain or distress that cannot be alleviated by analgesics
- Difficulty with ambulation (paralysis, fractures, trauma, etc.)
- Severe central nervous system signs (e.g., circling, rolling, persistent seizures or convulsions)
- Abnormal breathing (dyspnea) and cyanosis
- Body condition score of 2 (out of 5) or less (see FAQ for links to species-specific body condition scoring charts)
- Excessive weight loss (see institution-specific guidelines)
- Vomiting/diarrhea resulting in severe dehydration
- Tumor production specific endpoints (see FAQ for links to institution-specific guidelines)
- Other model-specific endpoints (please describe)

We do not expect or plan on peri-surgical infection. Thus, sign of infection such as skin necrosis and pus will be endpoints for euthanasia. We will also look for early signs of infection such as swelling and will check the animal's temperature if we suspect infection. 15% weight loss is also a criterion for euthanasia as it is indicative of the general well being of the animals being significantly compromised. If animals are nearing the weight loss threshold, we will consult facility veterinarians to supplement their diet.

We will monitor the animals for clinical signs of pain and distress such as lack of grooming, porphyrin staining, hunched posture, aggressive behavior and will evaluate the necessity of addressing their health status in communication with our facility veterinarians. We will administer methods alternative to pain medication as needed. These methods include diet supplementation and heat packs.

[redacted]

Please choose:

O Animals will be removed from the study and euthanized if any of the above clinical signs/conditions are found

◉ Some or all of the criteria listed above cannot apply to this study. Animals will be euthanized if the following criteria are met.

Indicate which criteria do not apply, and explain

Difficulty with ambulation will not be a criteria for euthanasia.

Functionally, some lameness or changes in gait are expected during the study. Non-weight bearing lameness or complete off-loading of the limb in stance or in gait is expected in the first two weeks after surgery, but this type of lameness for more than 14 days is indicative of an unexpected outcome and will invalidate our measurements of functional pain. Therefore, this is a criterion for terminating the study. However, this is a pilot study, so the course of recovery is not clear. We will notify the facility veterinarian in advance of initiating this study and will plan to assess the first cohort of animals together to determine if euthanasia is necessary.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. Endpoints**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

Migration Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. "Please describe other endpoints"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Tumor Form, Question 5. "Indicate other humane endpoints used"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

[redacted]

B. Moribundity and Mortality

The IACUC acknowledges that some studies may require moribundity (a clinically irreversible condition leading inevitably to death) or mortality (a fatal outcome) as an endpoint. The committee recommends that consideration be given to surrogate markers that can be utilized for a more humane endpoint, such as serial imaging or biomarkers that may permit the detection of experimental endpoints that precede the development of significant clinical signs, rather than allowing the animal to proceed to moribundity or mortality.

The use of death as an endpoint is strongly discouraged and requires scientific justification Rationale

1. Will this protocol include models with severe clinical signs expected?

O Yes ◉ No

2. Will this protocol use death as an endpoint?

O Yes ◉ No

C. Animal Transfer and Disposition

Select all that apply:

☑ Euthanasia or Terminal Procedure
☐ Transfer to another protocol at this institution (see FAQ for institution-specific guidelines)
☐ Transfer to another institution
☐ Release (field studies only)
☐ Animals may be considered for adoption.
☐ Animals may be considered for retirement.

D. Euthanasia Method

Euthanasia methods must be consistent with the AVMA Guidelines for the Euthanasia of Animals, 2013 edition. See FAQ for institution-specific guidelines/SOPs.

• A method must be indicated even if the protocol procedures are not terminal, for use in the event of an emergency.
• [redacted] protocols only: A secondary physical method to confirm euthanasia by carbon dioxide overdose or Isoflurane anesthesia overdose is recommended, but not required.

Species:

Rats (Rattus)

☑ Pentobarbital euthanasia solution (Euthasol, Fatal Plus, etc.); 100 mg/kg IP (0.22 mL/kg IP)
☐ Pentobarbital anesthetic overdose; 150-200 mg/kg pentobarbital IP

[redacted]

☐ Ketamine/xylazine anesthetic overdose; 240-300 mg/kg ketamine + 15-30 mg/kg xylazine IP
☐ General anesthesia, followed by non-survival surgery or exsanguination. *Please complete a procedure form to cover this method of euthanasia.
☐ Isoflurane anesthetic overdose; 5% isoflurane with secondary physical method.
☑ Euthanex chamber Units (CO2 chamber with no secondary physical method required)
☐ Carbon dioxide overdose (with secondary physical method)
☐ Cervical dislocation (without anesthesia) - animals
☐ Decapitation (without anesthesia) by rodent guillotine. *Proficiency must be observed and documented before this method of euthanasia may be performed independently by users.
☐ Hypothermia/cryoanesthesia, followed by secondary physical method - neonates
☐ Other

Will a sedative, tranquilizer, or anesthetic be administered prior to euthanasia?

◉ Yes ○ No

Provide the requested information for each agent (**Agent, Dose, Route**).

Agent	Dose	Route
Isoflurane	1-3% to effect (up to 5% for induction)	Inhaled

Migrated Data for [redacted] Protocols Only

This filed may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoint, Question 6.c. "If you plan to deviate from the approved [redacted] CCM Euthanasia SOPs, describe the euthanasia method"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

Housing [redacted]

I. HOUSING LOCATIONS

A. CCM Centralized Facilities
Select all applicable housing areas.

[redacted]

[redacted]

B. Investigator-Managed Facilities or Satellite/Laboratory Housing Areas

- Please note that permission to house animals in investigator-managed centralized facilities must be obtained from the appropriate satellite facility manager. See FAQ for contact information.
- All new satellite/laboratory housing areas must be inspected and approved by the IACUC and the Center for Comparative Medicine. Research cannot be conducted until the area has been inspected and notification of approval has been received.

Select applicable housing areas.

☐ [redacted]
☐ [redacted]

☐ Other IACUC approved satellite/laboratory housing area

☐ New satellite/laboratory housing area

C. Offsite Housing

All offsite housing locations must be inspected and approved by the IACUC and the Director, Center for Comparative Medicine. Animals may not be housed in a new location until it has been inspected and notification of approval has been received.

☐ Biomere
☐ Other

II. SPECIAL HANDLING, HUSBANDRY, OR HOUSING REQUIREMENTS

Will the animals on this protocol require any special handling, husbandry, or housing requirements? This includes anything outside of normal routine husbandry/handling services utilized by CCM, as defined in the species specific SOPs (e.g., alterations in bedding types, cage change frequencies, housing densities, special diets/fluids, deviations from currently approved IACUC policies, etc). See Species Specific Social Housing SOPs for more information.

Please discuss all special handling, husbandry, or housing requirements with CCM facility managers and/or veterinarians.

☑ No special housing or husbandry is required

☐ Breeding (i.e., delayed weaning requirements, harem breeding strategies, etc.)

☐ Immunocompromised

[redacted]

☐ Genetically modified animals (includes knock-outs, knock-ins, and transgenics)

☐ Specialized diet or fluid

☐ Alteration of cage / pen change frequency

☐ Alteration of light cycle

☐ Alteration of temperature and/or humidity

☐ Non-standard caging (e.g., metabolic cages, raised floor)

☐ Other

Exemptions from the Environmental Enhancement Program that are defined and approved by the IACUC Policy on Environmental Enrichment, Social housing and Exercise of Laboratory Animals do not need to be described in the protocol. A flow chart detailing the social housing policy is available to assist in the determination if planned single housing is covered by the policy.

☐ Non-social housing of social animals

☐ Withholding all cage, pen, or tank environmental enrichment

☐ Exemption from canine exercise program

If there are any relevant citations or other documents that are needed to support these special housing, husbandry, or handling requirements, please attach them to this form.

Anesthesia Regimen: Anesthesia Regimen: Ketamine/Xylazine for Rats

Please assign a label for this anesthesia regimen (e.g. Isoflurane Option, Surgical – Minor Procedure, Imaging Sedation, etc.). This label will be used in dropdown lists for other forms in this protocol.

Anesthesia Regimen: Ketamine/Xylazine for Rats

[redacted]

1. Enter the agents that will be used for this anesthesia regimen. Include sedatives, paralytic agents, and anesthetic reversal agents. Do not include local anesthetics or other drugs used for analgesia. **See FAQ pane for institution-specific formularies.**

Agent	Dose	Route	Frequency
Xylazine (initial)	5-10mg/kg	Intraperitoneal (IP) Injection	One dose
Ketamine (additional)	80-100mg/kg	Intraperitoneal (IP) Injection	Redose ketamine only if longer duration of anesthesia is needed.
Ketamine (initial)	80-100mg/kg	Intraperitoneal (IP) Injection	One dose provides 20 - 40 minutes of surgical-plane anesthesia

2. Are any of the agents listed paralytics?

○ Yes ◉ No

3. The IACUC requires that all anesthetics administered to any animal species be of pharmaceutical grade (USP grade), if that agent is available in pharmaceutical grade. Are all agents in this anesthetic regimen of pharmaceutical grade (USP grade)? **See FAQ for definition of pharmaceutical grade.**

◉ Yes ○ No

Species:
Rats (Rattus)

4. The adequacy or depth of anesthesia will be monitored by (select all that apply):

☑ Respiratory rate
☑ Toe pinch
☐ Corneal or palpebral (blink) reflex
☑ Other (please describe)

Continuous heart rate monitoring and pulse oximetry. Respiration rate is evaluated visually every 5-10 minutes.

5. How frequently will the depth of anesthesia be assessed?

See the Policy on Anesthesia and Analgesia for documentation guidelines for USDA-regulated and non-regulated species.

Animal will be monitored continuously with depth assessed at least every 15 minutes.

[redacted]

Anesthesia Regimen: Anesthesia Regimen: Isoflurane via Precision Vaporizer for Rats

Please assign a label for this anesthesia regimen (e.g. Isoflurane Option, Surgical – Minor Procedure, Imaging Sedation, etc.). This label will be used in dropdown lists for other forms in this protocol.

Anesthesia Regimen: Isoflurane via Precision Vaporizer for Rats

1. Enter the agents that will be used for this anesthesia regimen. Include sedatives, paralytic agents, and anesthetic reversal agents. Do not include local anesthetics or other drugs used for analgesia. **See FAQ pane for institution-specific formularies.**

Agent	Dose	Route	Frequency
Isoflurane via precision vaporizer	5%	Induction in chamber	To effect
Isoflurane via precision vaporizer	1-3%	Intubation or nosecone	Continuous

---

2. Are any of the agents listed paralytics?

○ Yes ◉ No

---

3. The IACUC requires that all anesthetics administered to any animal species be of pharmaceutical grade (USP grade), if that agent is available in pharmaceutical grade. Are all agents in this anesthetic regimen of pharmaceutical grade (USP grade)? **See FAQ for definition of pharmaceutical grade.**

◉ Yes ○ No

Species:

Rats (Rattus)

4. The adequacy or depth of anesthesia will be monitored by (select all that apply):

☑ Respiratory rate
☑ Toe pinch
☐ Corneal or palpebral (blink) reflex
☑ Other (please describe)

Continuous heart rate monitoring and pulse oximetry. Respiration rate is evaluated visually every 5-10 minutes.

5. How frequently will the depth of anesthesia be assessed?

See the Policy on Anesthesia and Analgesia for documentation guidelines for USDA-regulated and non-regulated species.

[redacted]

Animal will be monitored continuously with depth assessed at least every 15 minutes.

Procedures: Identification Ear Tag for Rats

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Identification Ear Tag for Rats

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Ear Tag

[redacted]

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

To be defined. Ear tag identification will occur in the same room rats are housed in.

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

○ Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required ○ Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

○ Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

a. Animals ≥ 2 weeks of age are manually restrained by scruffing

[redacted]

b. A metal tag of appropriate size for the species with a unique identification number is attached to the ear of the animal.

c. Tags will be washed in alcohol or sterilized prior to application and are applied using a commercial Animal Tag Applicator.

d. Tags will be applied to the inner lower 1/3 of the pinnae, just above the skin fold taking care to avoid the firm cartilaginous portion of the lower ear.

e. Wire suture scissors will be used to remove tags if needed

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

Animals will be monitored immediately after the procedure to ensure no discomfort or adverse effects. The tag will be checked regularly to ensure proper placement.

---

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

[redacted]

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Weight Monitoring

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

[redacted]

Weight Monitoring

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
weight monitoring

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

[redacted]

◉ Not painful/not required

O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Place rat in a bowl on the scale and record the weight until the reading is steady. Return the rat to its home cage.

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

Weight will be measured on pre-op day 1 and POD 0, 1, 3, 5, 7, 9, 11, 13, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56 (approximately every other day, see flowchart).

---

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

[redacted]

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Procedures: Tail Vein Nick for Rats

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of

[redacted]

definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Tail Vein Nick for Rats

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Tail Vein Blood Extraction

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food

[redacted]

☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Frequency of blood collection: We will collect blood on preoperative day 1 and postoperative days 1, 3. 7, 14, 28, 42, and 56.

Volume of blood per blood draw: 120 ul

For a 24-hour period: "The easiest way to determine the total blood volume for an animal is to follow the general principle that total blood volume is equivalent to 6% of the animal's body

weight."

TOTAL BLOOD VOLUME (mls) = BODY WEIGHT (grams) X 0.06

TOTAL BLOOD VOLUME (mls) = 300 X 0.06 = 18 mls

120 ul collected in 24 hours = 0.12 ml

[redacted]

0.12/18 X 100 = 0.66% of total blood volume (less than 6%, acceptable amount)

For a 14-day period: "Most healthy animals can tolerate losing up to 15% of their total blood volume over a two-week period."

TOTAL BLOOD VOLUME (mls) = BODY WEIGHT (grams) X 0.15

TOTAL BLOOD VOLUME (mls) = 300 X 0.15 = 45 mls

5X120 ul collected in 14 days = 0.6 ml

0.6/45 X 100 = 1.33% of total blood volume (less than 15%, acceptable amount)

Method:

1. The tail can be vasodilated by working under a heat lamp or placing the tail in warm water (37oC).

2. Animal will be restrained with plastic rodent restrainer or firm physical restraint.

3. The lateral tail vein will be nicked about 2-3 mm from the tip with a sterile needle or lancet.

4. Blood will be collected with a capillary tube or collection tube.

5. After collection, bleeding will be stopped by applying gentle pressure to the area using gauze; a stypic agent will be applied if needed.

6. The scab or clot will be removed using dry gauze for repetitive samples. If another nick is necessary, it will be made above the original nick, moving closer to the base of the tail.

7. The total volume of blood collected will not exceed the maximum permitted by the [redacted] IACUC per the Policy on Blood Collection.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with

[redacted]

CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

Animals will be observed immediately post procedure to ensure bleeding stops. Additional observations are in accordance with the experimental group for the animal as described elsewhere in the protocol.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

[redacted]

◉ Yes O No O Not applicable

Procedures: Core temperature monitoring

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Core temperature monitoring

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Rectal temperature measurement

[redacted]

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

The core temperature measurement will be made by a rectal thermometer while rat is in a plexiglass restrainer (see restraint section). This procedure will only be done if infection is suspected. It has been confirmed with the facility veterinarian that measurement of systemic body temperature could contribute

[redacted]

to identification of systemic infection or sepsis.

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

Temperature measurements will be taken if we suspect systemic infection. The indicators would be drastic changes such as significant weight loss, lethargy, non-grooming, pus etc.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

[redacted]

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: von Frey measurement for pain response

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
von Frey measurement for pain response

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

[redacted]

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Von Frey Test

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

[redacted]

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

The animals will be acclimatized to the procedure room in their cages for at least one hour. Animals will be placed individually on an elevated von Frey cage in plastic enclosures and acclimatized for about 15 minutes. An electronic von Frey probe will be applied to the rat's paw and will measure the load at which a withdrawal response is observed. Surgical and non-surgical limbs will be tested. Five measurements will be performed for each hindpaw. After the procedure, the animals will be placed back in their cages.

von Frey acclimitization will take place on pre-op days -4, -3, and -2. von Frey measurements will performed pre-op on day -1 and post-op days 1, 3, 7, 14, 28, 42, and 56

---

a. Is this a tumor production procedure?

○ Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

We will follow general monitoring plan (daily). There is no specific follow-up needed for von Frey measurements. The animals are not expected to be in additional distress because of these measurements.

---

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Suture removal

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

[redacted]

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Suture removal

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Suture removal

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
To be defined. Suture removal will occur in the same room rats are housed in.

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

[redacted]

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

The rat will be restrained and sutures will be removed.

Suture removal will be performed at 7-10 days post-op.

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

[redacted]

Does not apply for suture removal.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Tibial Fracture and Plate Fixation

[redacted]

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Tibial Fracture and Plate Fixation

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

O Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation,

◉ Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive

[redacted]

percutaneous biopsy	tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Fracture, Creation/Repair

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site
☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
☐ Preparation of aquatic species

[redacted]

☑ Draping of the animal
☐ Other

---

b. Preparation of the Surgeon

☑ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☐ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave
☐ Chemical sterilant
☑ Ethylene oxide sterilizer
☑ Glass bead sterilizer (Only for sterilizing between animals)
☐ Plasma sterilizer
☐ Purchased sterile from vendor
☐ Other

---

2. Indicate other preoperative preparation:

☑ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Anesthesia Regimen: Isoflurane via Precision Vaporizer for Rats

---

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes O No

[redacted]

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenorphine hydrochloride	0.05 mg/kg	SC or IP	Single dose	30 minutes before surgery

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Procedure: Tibia fracture with titanium "Y" shaped plates and titanium alloy screw in tibia

Description: Leg fur will be shaved on both legs. Skin on the surgical limb (right leg) will be scrubbed with Betadine. A skin incision will then be made using surgical scissors and then the metaphyseal transverse osteotomy of the proximal tibia will be performed. A 1 mm osteotomy gap, 5 mm distal to the tibia plateau, will be created using an oscillating, sagittal saw. The fracture fixation will be conducted on the ventromedial aspect of the tibia with a 2.3 cm long and 0.7 mm thick 5-hole Y-plate. 4 craniofacial screws (1.1 mm diameter, ~6-8 mm length) will be used to secure the plate (see flowchart for surgical diagram). After plate fixation, the muscular structure will be closed with a running subcuticular 5-0 Vicryl followed by a running 5-0 Vicryl subcuticular suture to close the skin. The estimated duration of the procedure is 30 minutes, with a maximum procedure time of 40 minutes. All plates, screws, and surgical instruments/supplies used will be sterile.

Monitoring plan / criteria used to determine if animals are stable: The animals will be maintained on a heating pad (circulating water) during surgery and until recovery from (isoflurane) anesthesia. Respiration rate is regular, fully awake after anesthesia.

a. Is this a tumor production procedure?

O Yes ◉ No

[redacted]

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☑ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

heating pad or lamp during recovery from anesthesia

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☐ Animal can sit upright (NHPs)
- ☑ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

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1. Indicate the frequency of post-procedural observations

Twice daily for 3 days, then daily.

2. Will post-operative/post-procedural analgesics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenorphine hydrochloride	0.05 mg/kg	SC or IP	every 6-12 hr (standard schedule will be every 8 hours)	3 days

[redacted]

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Gait Recording

Complete this form for each procedure/surgery to be performed.

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Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

[redacted]

Gait Recording

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Gait analysis

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

[redacted]

◉ Not painful/not required

O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Rats will be taken out of the cage and allowed to walk in a clear, open, plexi-glass corridor (dimensions 10" dia x 6" height x 30" length).

Gait will be digitally recorded using a digital camera.

Each rat will remain in the gait arena for 10 minutes.

Afterwards, the rat will be returned to its cage.

Gait acclimatization will take place on pre-op days -4, -3, and -2. The gait recording will be performed on pre-op day -1 and post-op days 1, 3, 7, 14, 28, 42, and 56

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

[redacted]

We will follow the general monitoring plan. Post-procedure observation specific to the gait recording is not necessary.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: X ray Imaging

[redacted]

Complete this form for each procedure/surgery to be performed.

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Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
X ray Imaging

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Imaging, X-Ray

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

[redacted]

2. Indicate other preoperative preparation:

☑ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Anesthesia Regimen: Isoflurane via Precision Vaporizer for Rats

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Rats will be anesthetized using isoflurane. Rats will be transferred to an xray machine and their hind legs will be secured in place with surgical tape. The x ray will be taken and then the rat will be removed immediately and returned to its cage. The rat will be in the X ray machine for less than 10 seconds. X ray will be performed on POD 1 to ensure correct placement of the plate.

a. Is this a tumor production procedure?

O Yes ◉ No

[redacted]

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☑ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

warming pad or heat lamp

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☐ Animal can sit upright (NHPs)
- ☑ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

Animal will be monitored continuously until it is ambulatory. Then it will be monitored twice a week.

---

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia

[redacted]

cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Euthanasia

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the

[redacted]

operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Euthanasia

A. Procedure Type

1. What is the type of procedure?

○ Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

○ Survival ◉ Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Euthanasia

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] for Euthasol or [redacted] for CO2 units

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

[redacted]

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Anesthesia Regimen: Isoflurane via Precision Vaporizer for Rats

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Rats will be euthanized by pentobarbital (200 mg/kg IP) (Euthasol) after isoflurane sedation in [redacted]. Rats can also be euthanized using CO2 following guidelines posted besides the CO2 tank in [redacted]. 8 rats will be euthanized on post-op day 14, 8 on day 28, 8 on day 42, and the remaining 8 on day 56 (see flowchart). We will euthanize at different time points so that we can perform ex vivo microCT to monitor healing of the bone.

a. Is this a tumor production procedure?

O Yes ◉ No

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Static Weight Bearing

[redacted]

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Static Weight Bearing

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Gait analysis

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Rats will be placed in a plexi-glass enclosure over a sensor mat (TekScan). The rat will remain on the sensor (force plate) for up to 4 minutes while the force the animal is applying on each limb is measured by the sensor. We have chosen a 4 minute time period because most of the time, the rats are walking around, and our analysis requires a 10 second stationary period over which we can average the force on each limb. To clarify, we are measuring force applied to each limb so that we can detect when the animal is offloading a particular limb (NOT the time it is spending on each limb). The TekScan sensor we use is made specifically for small animal weight bearing.

[redacted]

Weight bearing acclimatization will take place on pre-op days -4, -3, and -2. Static weight bearing will be performed on pre-op day -1 and post-op days 1, 3, 7, 14, 28, 42 and 56.

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

We will follow the general monitoring plan. Post-procedure observation specific to the gait recording is not necessary.

---

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

[redacted]

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Controlled Substance and Non-Pharmaceutical Grade Substance

A. Controlled Substances

1. Are any of the agents (anesthetics, analgesics, test agents, etc.) used in this protocol DEA/Federally Controlled Substances?

◉ Yes O No

a. Indicate where DEA/Federally Controlled Substances will be stored. If your license is pending or if a secure location is not yet identified, please enter "TBD" in the Room field.

Building:

[redacted]

b. Room:

[redacted]

B. Non-Pharmaceutical Grade Substances

Both OLAW and AAALAC provide guidance regarding the use of non-pharmaceutical grade compounds in laboratory animals.

Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results. However, it is frequently necessary to use non-pharmaceutical-grade substances such as investigational substances, veterinarian- or pharmacy-compounded substances, and/or Schedule I controlled substances to meet scientific and research goals.

A listing of pharmaceutical-grade drugs and biologics is available through the FDA database.
- The Orange Book is the reference for FDA-approved human drugs.
- The Green Book is the reference for FDA-approved veterinary drugs.

1. Are all substances to be administered to animals of pharmaceutical grade?

Examples of non-pharmaceutical grade substances include:

- Anesthetics and analgesics (e.g., Avertin)
- Euthanasia compounds (e.g., pentobarbital)
- Diluents and/or vehicles (e.g., DMSO, methyl cellulose)
- Test compounds

◉ Yes O No

Restraint and Device Acclimation

The Guide for the Care and Use of Laboratory Animals defines physical restraint as the use of manual or mechanical means to limit some or all of an animal's movement for the purpose of examination or experimental manipulation. Sedatives or anesthetics may be used to immobilize animals for the performance of non-painful procedures that might otherwise be painful or distressful to the animal.

Restraint

◉ Animals will undergo restraint as part of this research

1. Provide justification for the use and duration of restraint.

For tail bleed.

2. Will the animals be conscious or sedated during the restraint?

◉ Conscious O Sedated

3. Indicate the type of restraint that will be used.

☐ Manual restraint

☑ Mechanical

[redacted]

Select all that apply.

- ☑ Rodent plexiglass, metal, or Bowman style restrainer
- ☐ Rabbit plexiglass or metal retrainer
- ☐ Full body sling
- ☐ NHP chair
- ☐ Stereotaxic device
- ☐ Squeeze cage
- ☐ Other

a. Indicate the duration of mechanical restraint (select all that apply).

- ☑ Routine mechanical restraint for less than 15 minutes
- ☐ Restraint duration longer than 15 minutes, but less than 4 hours
- ☐ Restraint duration longer than 4 hours

b. Indicate the frequency of mechanical restraint.

The rat will be placed in a plexiglass restraint with the tail accessible. The lateral tail vein will be lanced and 150 microliters of blood collected. Pressure is applied until hemostasis is achieved. The procedure will take 2-5 minutes to collect blood.

c. Describe the methods used to train and acclimate animals to the mechanical restraint device.

The animal will be placed in a commercial restrainer for the absolute minimum time to lance the tail vein and collect blood.

d. Describe the plans for monitoring and care of the animals during the periods of mechanical restraint.

No additional monitoring is necessary.

The Principal Investigator is responsible for assuring that:

· Veterinary care will be provided if lesions or illnesses are observed

· The purpose and duration of restraint will be communicated to all personnel involved in the study.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Anesthesia Regimen, Label**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it contains details useful in answering the **Indicate the anesthesia regimen or sedative that will be used**, question above.

Device Acclimation

[redacted]

◉ This research includes devices to which animals must be acclimated, e.g., jackets/tethers

1. Indicate the type of device that will be used (select all that apply):

☐ Jacket, Harness, or Tether
☐ Behavioral Testing Device
☑ Other devices

Describe other types of devices:
Rodent plexiglass restrainer

2. Indicate the frequency and duration of the device use.
2-5 minutes each time, daily

3. Describe the methods used to train and acclimate animals to the device.
Rodents will be placed and kept in the device for 2-5 minutes for up to 1 week before surgical procedure, similar to other procedures on the protocol requiring acclimatization

4. Describe the plans for monitoring and care of the animals while they are in the device.
No additional monitoring is necessary

Transportation

The transportation of animals must conform to IACUC policy and the Animal Welfare Act, as applicable.

1. Will live animals be transported to facilities outside the institution?

O Yes ◉ No

2. Will animals be moved within or between institution facilities? This includes moving animals between housing areas and laboratory or imaging areas.

◉ Yes O No

[redacted]

a. Will animals be moved between Biosafety Level 2 (BSL2) housing and laboratory or imaging areas?

O Yes ◉ No

b. Select the option that applies:

☐ Transfers will be performed by CCM
☑ Transfers will be performed by protocol study staff following institutional SOPs and/or guidelines (see FAQ for links to institution-specific SOPs/guidelines).
☐ Other (including any animal transport not covered by institutional SOPs or guidelines)

Initial Survey

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

In accordance with federal regulations and hospital policies, all animal research conducted at or funded through the [redacted] must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of the study. This policy applies to any vertebrate animal used for any type of research, teaching, or testing. The IACUC has the sole authority to approve, require modifications (in order to secure approval), or withhold approval of research protocols involving the use of animals at the selected Institution. Protocols can be approved for a maximum of three years, subject to satisfactory annual reviews where required. The IACUC also must review and approve **in advance** any changes or modifications to previously approved protocols.

Principal Investigator Eligibility: Please note that you must meet the eligibility requirements set by your institution's IACUC in order to serve as the principal investigator (PI) for an animal research protocol. See FAQ for links to institution-specific guidelines.

The questions below will help to identify if an IACUC protocol must be submitted to your institution's IACUC for your research project.

Please enter the full title of the study.
Tibia fracture model with plate fixation

A. At which Institution will the research be conducted?

◉ [redacted] or [redacted] O [redacted] O McLean O Other Institution

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[redacted]

B. The proposed research project will involve the following:

◉ The entire animal research protocol will be conducted at the selected institution.

O Only a portion of the animal research project will be conducted at the selected Institution. This includes, but is not limited to, housing, surgery procedures, behavior assessments, imaging sessions, etc.

If the IACUC grants approval, it will oversee only the research component that is performed at your institution. Any research component(s) conducted at an outside institution will be conducted under the auspices of that institution's IACUC.

For the protocol to remain active, the investigator must submit satisfactory IACUC annual progress reports (if required), as well as provide annual documentation of the relevant outside IACUC approvals.

For more information, please refer to the IACUC website for your institution:

- [redacted]

C. The proposed study involves the use of:

◉ Any live animal (ie. mouse, rat, rabbit, dog, cat, swine, sheep, nonhuman primate, etc)

O Animals tissues, products, or blood (including whole dead animal), not otherwise approved by the IACUC as part of the investigator's own animal research protocol.

Attachments

Name	Mode
Flowchart 20190326 (Flowchart)	Electronic
Point by Point Response_Initial Review_20190314 (Point by Point Response)	Electronic
Point by Point Response_Committee Review_20190326 (Point by Point Response)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic