UCI Office of Research
Research Management System

Animal Use Protocol

Title

General Information

Project Overview

Provide a non-technical, lay-language summary of your project.

Uncheck this box to remove all text from the box below

The inner ear is comprised of the auditory and vestibular systems, which provide the brain hearing and balance information. Sensory cells of the vestibular system detect movements of the head and reflexively signal eye and limb muscles to contract and relax appropriately to stabilize the visual field and maintain balance. These reflexes enable us to read while walking or riding in the car, and without a functional vestibular system, involuntary eye movements to keep the visual field stable during head movement would not occur. Patients with vestibular problems often suffer from debilitating issues with balance, vertigo, and oscillopsia, a condition in which the visual field is unstable and moves uncontrollably with any movement of the head.

Scientists have recently investigated the benefits of placing an implant into the vestibular organs of animals to provide electrical stimulation and restore vestibular signaling to the brain. Moreover, in one institution, a human subject with a common vestibular disorder has received an implant. In these studies, investigators have engineered implants designed to be placed into the bony labyrinth of the inner ear. in the auditory system that stimulation of the nerve itself, rather than the sensory organ, provides superior and broader sensory stimulation. Accordingly successfully use electrodes to stimulate vestibular nerves of Guinea pigs to determine if can elicit specific eye movements. We will continue this line of research to determine whether or not chronic implantations of these electrodes in the vestibular nerve of a cat can result in stable long-term vestibular nerve stimulation. The research will provide practical knowledge concerning optimal ways to stimulate the vestibular system and to improve our ability to restore balance function.

Study Characteristics

Surgery

Types of Surgery

Terminal (animals will not recover from anesthesia)

Survival

Major

Will MULTIPLE major survival surgeries be performed on a SINGLE animal?

No

Prolonged Restraint (without sedation or anesthesia)

Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for research purposes.

Scientific Justification for Prolonged Restraint

Uncheck this box to remove all text from the box below

<u>Screw Head Fixation</u> (GUINEA PIGS ONLY)

In the vestibular research field, rhesus monkeys are the primary animal model for the testing of vestibular system implants. are the main laboratories studying vestibular implants, and in each of these labs, the stimulating implants are surgically placed, and the animals are allowed to recover for days to weeks. Subsequently, monkeys are tested while alert and awake with their heads rigidly restrained (e.g.

This is because the only objective and measurable method to test vestibular nerve activity is to stimulate the vestibulo-ocular reflex, which allows the eyes to move involuntarily and accurately with all head movements to maintain the visual area of interest on the fovea of the retina. Measurements of the velocity, direction, and gain of the eye movements have to be exceedingly precise, and accordingly the head of the animal must be fixed. It is an exciting and important area of research that has merited at least five grants in the past two to three years alone .

With our experience with we believe we can introduce a novel method to stimulate the vestibular system – by placing a multi-channel electrode array into the vestibular nerve. We'd like to take the elementary steps to prove the principle. to stimulate the vestibulo-ocular reflex in Guinea pigs were unsuccessful because underestimated the extent to which brainstem reflexes are suppressed under anesthetic sedation. This is why all other national labs perform their animal experiments on alert, awake animals and with their heads restrained.

You MUST provide details about the prolonged restraint in the Procedures Tab:

Animal Biosafety Level
ABSL-1

Will animals be physically restrained for more than brief periods without the use of sedation or anesthesia?
Yes

Will paralytic agents be used in live animals under this protocol?
No

Will surgical procedures be performed under this protocol?
Yes

Type of surgical procedure(s) to be performed (check all that apply):
Major

Will animals recover from surgical procedures (survival surgery)?
Yes

Will multiple survival surgeries be performed on a single animal?
No

Project Continuation

Is this application a three-year de-novo renewal of a previously approved protocol?
Yes

Total # of animals used in the last 3 years
Adverse Events & Unanticipated Problems
Uncheck this box to remove all text from the box below

None

Progress Summary
Uncheck this box to remove all text from the box below

Progress on this protocol has stalled due to surgical/anatomic constraints of the animals. We have recently established a collaboration with the [redacted] lab to study vestibular nerve stimulation, and we will likely revisit this protocol (and perhaps submit a modification to add a new species) soon.

Justification for Continuing the Project
Uncheck this box to remove all text from the box below

There is considerable clinical work in vestibular stimulation, and here at UC Irvine we do have the expertise and resources to make contributions to this line of work. We will be collaborating with a [redacted] vestibular nerve stimulator, [redacted] bring their work to animal studies.

List the previously approved protocol number

[redacted]

Funding Source	Funding Status	Award/Proposal #	Billing Account #

Funding & Billing Information

Other Funding Sources (not captured in table above)
Uncheck this box to remove all text from the box below

Has the project undergone peer-review by an extramural sponsor/funding agency (e.g., NIH study section)?
No

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

Species

Cat
Guinea Pig

Cat

Species Justification

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

Cats have auditory and vestibular systems very similar to that of humans. The cat vestibular system is physically more accessible, with larger anatomy conducive to surgical insertion and chronic placement of our electrodes. The Guinea pig anatomy is too small for surgical implantation of our Microprobes electrode arrays. Even with the smallest electrode arrays, accurate placement and confident security of the array within the Guinea pig nerve will be unacceptably challenging, unpredictable and unreliable. All study team members have extensive experience using Cats for acute and chronic physiological experiments involving stimulation and implants of the inner ear.

A smaller animal (e.g., rat or mouse) would not be acceptable because access and stimulation of the vestibular nerve and video documentation of their eye movements would be difficult or impossible. A more sophisticated animal (e.g., Primate) would not be justified for these experiments at this time because the Guinea pig and Cat vestibular and ocular systems are sufficient for the proposed study of the vestibulo-ocular reflex. The Cat must be used because of physical size limitations of Guinea Pig anatomy, as well as limitations in detecting the specific directionality of the stimulated eye movements that can readily be addressed in Cats.

Animal Characteristics

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

N/A

Phenotypic Abnormalities or Special Health Conditions
Uncheck this box to remove all text from the box below

N/A

Additional Information about Species/Strains
Uncheck this box to remove all text from the box below

N/A

Guinea Pig

Species Justification

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

Guinea pigs have auditory and vestibular systems similar to that of humans. The guinea pig vestibular system is unusually accessible, providing relatively easy access for vestibular nerve stimulation. All study team members have extensive experience using Guinea pigs for acute physiological experiments involving stimulation and implants of the inner ear.

A smaller animal (e.g., rat or mouse) would not be acceptable because access and stimulation of the vestibular nerve and video documentation of their eye movements would be difficult or impossible. A more sophisticated animal (e.g., Primate) would not be justified for these experiments at this time because the Guinea pig and Cat vestibular and ocular systems are sufficient for the proposed study of the vestibulo-ocular reflex.

Animal Characteristics

List the specific strains that will be used.

Phenotypic Abnormalities or Special Health Conditions
Uncheck this box to remove all text from the box below

N/A

Additional Information about Species/Strains
Uncheck this box to remove all text from the box below

N/A

Rationale & Alternatives

Search Results

Date of Search	Time period from	Time period to	Database	# of Results	Keywords
Oct-11-2017	Oct-11-2014	Oct-11-2017	PubMed	141	vestibular nerve prosthesis OR vestibular nerve implant OR vestibular nerve stimulation
Oct-11-2017	Oct-11-2014	Oct-11-2017	Web of Science	285	vestibular nerve prosthesis OR vestibular nerve implant OR vestibular nerve stimulation

Database Searches

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

The literature search reveals many studies investigating the movements of the eye with vestibular system stimulation with broad, galvanic current non-specifically delivered to temporal bone, or with targeted current delivered to the semicircular canals. The latter stimulation design has been studied in a wide range of animals, including Guinea pigs, chinchillas, and rhesus macaque monkeys, among others. Moreover, the use of this design – stimulation of the semicircular canals – has also been reported in a human subject in early 2014. A modified cochlear implant with three electrode arrays was implanted into the three semicircular canals of a patient with uncontrolled Ménière's disease. Although the surgery was successful, the audiometric and balance outcomes for the patient failed to meet expectations. These results highlight both the clinical interest in providing vestibulopathy patients with rehabilitative options and the need for new and better ways to target and effectively activate the vestibular system.

and accordingly we are uniquely positioned to investigate vestibular system stimulation with this device. These experiments are designed to provide "proof-of-concept" results in order to confirm and document that the electrode array can activate the vestibulo-ocular reflex, and consequently, these experiments will require a number of minimal animals. There is essentially no scientific overlap between the proposed work and previous studies. labs working on vestibular system stimulation, and close communication between those groups prevents duplication of efforts.

Update 10/11/17: The literature review reveals that there is considerable continued work on the CLINICAL vestibular implant, but little new progress in preclinical animal work. The groups, both of whom use monkey models, have not published in this time period. The group does have a new publication in 2016 also using monkeys. However, this is the same approach (inner ear stimulation) that they have published on many times, and is NOT the approach we are investigating (intraneural stimulation). Accordingly, there is little chance at unnecessary duplication of other published work or applicable use of alternative methods or refined procedures.

Other Sources Used to Consider Alternatives

Replacement

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

Previous in vitro experiments have yielded useful information about the microanatomy and electrophysiology of the vestibular end organs, but these experiments cannot examine the nature of brainstem processing of vestibular information addressed by the present experimental questions. In vitro procedures offer little for the multi-level systems questions that are raised.

Reduction

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

Our numbers reflex the range of number of animals other investigators have been required to utilize to obtain sufficient data and achieve statistically significance. We will process our data as we proceed with our experiments and will strive to use the minimal number of animals to achieve our research goals.

Refinement

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

Members of our study team have decades of experience with working with animals in research, and in particular, with Guinea pigs and Cats. These proposed experiments are both short in duration (~4 hours) and terminal in nature. We will follow standard protocols to ensure that the animals are well-anesthetized during the entire course of each experiment.

Study Segments

Experimental Design	Species
Study Segment	Guinea Pig,Cat

Study Segment

Species

Cat
Guinea Pig

Experimental Design Summary

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

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

Experiment A. Video recording of ocular movements with stimulation of the vestibular nerve with a penetrating electrode array

1. Rationale:

The vestibular system is comprised of 5 independent sensory organs (3 semicircular canals and 2 otolithic organs) which collectively detect all movements of the head in three-dimensions, including angular (rotational) acceleration and linear (gravitational) acceleration. Other investigators studying vestibular system stimulation and implantation have uniformly directed their efforts at accessing and stimulating the individual semicircular canals and/or otolithic organs, which are confined within the complex inner ear labyrinth of the temporal bone, in the hopes of providing the vestibular nerve and, ultimately, the brain with adequate and minimally-specific sensory information. Designing and placing such stimulating electrode arrays for each sensory organ require substantially added engineering complexity and surgical difficulty. [redacted] described numerous and significant audiometric benefits of stimulating the cochlear nerve with a penetrating electrode array which allows for direct contact of the electrode surface with excitable neural tissue. Compared to the conventional cochlear implant in which the stimulating electrode array is placed into the sensory organ, direct stimulation of the cochlear nerve provided broader frequency range access, decreased levels of current required to activate nerve fibers, improved fidelity of signal to downstream neural pathways, higher selectivity of stimulated nerve fibers, and reduced interference between simultaneously-stimulated adjacent electrodes.

We aim to conduct a 'proof-of-concept' experiment to assess the ability of the penetrating electrode array used in the above published experiments to stimulate the

vestibular nerve and vestibulo-ocular reflex. Simply put, we will (1) surgically access the vestibular nerve, (2) place the penetrating electrode array into the vestibular nerve and activate the individual electrodes of the array, and (3) look for and video document the consequent movements of the eyes, also known as nystagmus. The video documentation will allow us to quantify the speed and directionality of the eye movements and determine if we can (1) independently stimulate nerve fibers originating from each of the five vestibular sensory organs, and (2) modulate the intensity of nystagmus with varying characteristics of electrical delivery.

2. Groups and procedures:

- A. Pilot experiments: 5 Guinea pig; 2 cats. This is to confirm that we can stimulate the vestibulo-ocular reflex and elicit nystagmus.
- B. Quantitative evaluation of parameters: 30 Guinea pigs; 8 cats. These experiments will be completed following the purchase and setup of a video oculography system to objectively track and qualify the direction and speed of the electrically-evoked nystagmus.

3. Justification of animal numbers:

Experiments consist of recording eye movements during electrical vestibular stimulation. The experimental stimulus parameters of interest include current level, pulse rate, time interval between pulses on pairs of channels, and electrode configuration (i.e., location of active and return electrodes). A pilot experiment will be conducted to work out technical challenges and to identify ranges of stimulus parameters appropriate for later systematic study. Based on the pilot results, we will identify a list of parameter values to be tested across multiple animals. The video documentation of various speeds and directions of the eye movements will provide the material needed to design a system to accurately measure electrically-evoked nystagmus. We include an additional 2 Guinea Pigs in Group B to allow for occasional failed experiments.
previously published work in guinea pigs presented data from 8 to 20 animals, which does not include the approximately equal number of animals used to develop the stimulation and recording procedures and to explore the parameter spaces to establish appropriate ranges of values to test in detail quantitatively. We anticipate needing 12-17 Guinea pigs to provide usable and publishable data, and accordingly we are requesting the use of 25 to 35 guinea pigs for this protocol.
previously published work in cats presented data from 4-6 cats. We anticipate needing

about 6 animals to provide usable and publishable data, and accordingly we are requesting the use of 8 cats for this protocol.

4. Sequence and Timing:

Terminal experiments:

- i. Induction of anesthesia using ketamine and xylazine (anesthesia for Guinea pigs to be maintained for the remainder of the experiment with supplemental doses of ketamine alone). For cats, anesthesia will be maintained for the remainder of the experiment with doses of Nembutal.
- ii. Fixation of the skull in a head-holding device.
- iii. Application of topical anesthesia to the eye followed by application of superficial marker onto the cornea via a tissue adhesive.
- iv. Craniotomy and implantation of the penetrating electrode array into the vestibular nerve.
- v. Wound closure via sutures or staples.

---- Steps i through iii typically take about 2 hrs ----

- v. The animal will be partially reversed with atimpamezole or yohimbine, and the animal allowed to wake up from sedative anesthesia. This may take 30-90 minutes. The animal remains in head fixation. Again, this is for the Guinea pigs only.
- vi. Video recording of eye movements in response to electrical stimulation through the penetrating electrode array. The data collection phase typically lasts about 30-60 minutes.
- vi. Euthanasia with lethal dose of barbiturate. Some animals will be perfused trans-cardially with paraformaldehyde, and the vestibular nerve harvested for reconstruction of electrode track.
- vii. End of experiment.

5. Procedural endpoint: About 4 hours after initial induction of anesthesia, data collection will be terminated and the animal euthanized. A subset of the animals will be perfused transcardially with paraformaldehyde to preserve the vestibular nerve for histological reconstruction of electrode tracks.

Experiment B. Chronic stimulation of the vestibular nerve via an implant.

1. Rationale: We propose to test the safety of long-term implantation and weekly stimulation of the vestibular nerve fibers by implanting an intraneural array chronically and monitoring effects of continuous stimulation for up to 3 months. We will be

particularly interested in any physical trauma to the nerve resulting from the implanted array and any effects, either positive or negative, on transmission through the vestibular nerve to eye movement. Functional effects of chronic stimulation will be assessed by video recording of eye movements. The day-by-day maintenance or drift of voltage thresholds will serve as a monitor of the long-term stability of chronic vestibular nerve implantation and stimulation. Also, after the period of chronic implantation, we will test each animal in a terminal experiment. The terminal experiment, under anesthesia, will be largely identical to the acute experiments and will involve a battery of tests in the awake and body-restrained cat for electrically-evoked nystagmus. The purpose of the terminal study is to test whether or not the precision and extent of stimulation that we have observed in acute experiments is preserved during and after chronic stimulation. Anatomical effects will be monitored by histological examination of the nerve. Based on previous experience with implantation of similar electrode arrays in nervous tissue, we hypothesize that the vestibular nerve implant will be tolerated well by the nerve, but that remains to be demonstrated.

2. Groups and procedures: Chronic implantation and stimulation: 6 cats. The purpose of this group is to test safety and efficacy of 3 months of weekly stimulation. These animals will be implanted with chronic vestibular -nerve stimulating arrays. They will receive pulsatile electrical stimulation once per week. At regular intervals, the animals will be sedated with acepromazine, and the anesthesia will then be reversed in the body-restrained cat in order to measure ocular activity. That testing will take place once per week for 3 months. After 3 months of chronic stimulation, each animal will be studied under anesthesia in a terminal experiment.

3. Justification for animal numbers: This work involves development of new surgical and testing procedures and application of new custom implants. This is challenging work, and we need to allow for the possibility of implant design modifications and some failures. In the chronic group, we have allotted up to 8 animals to allow for good quantitative data from 6 animals after a likely sizeable number of early design modifications and failures.

4. Sequence and timing:

- Day 1, Aseptic surgery to implant a chronic stimulating array: (i) induction of anesthesia with ketamine followed by isoflurane; (ii) exposure of the vestibular nerve as it exits the brainstem by the lateral

hemispheres of the cerebellum; (iii) implantation of chronic vestibular nerve stimulating electrode array; (iv) recovery from anesthesia.

- Day 7 (or after sufficient recovery), measurement of stimulation thresholds followed by onset of chronic stimulation: (i) sedation with a acepromazine; (ii) natural wakeup from anesthesia in a body-restrained cat in order to conduct non-invasive video recording of eye movement to electrical vestibular nerve stimulation, which is needed to demonstrate viability of the vestibular nerve implant and to select current levels for chronic stimulation; iii) beginning of chronic stimulation. The cat will be restrained loosely with a harness attached to the platform, in a manner identical to a previously –approved IACUC protocol. The harness will permit the cat to stand, sit, or lie down, but will prevent it from turning around completely (which would damage the stimulating cable) or from jumping off the platform.
- Beginning Day 9, routine data collection, ~1 hr, once per week for 12 weeks:: (i) initial sedation with acepromazine ; (ii) parametrically varying electrical stimulation through the vestibular nerve electrodes with video recording of eye movement. Stimuli will consist of single charge-balanced biphasic electrical pulses, initially cathodic, 41 or 82 µs per phase. The responses will be obtained with stimulus charge levels of 26 to 41 nC per phase. Pulse durations will be 200 microseconds.
- Day ~90, termination of experiment. The animal will be administered a lethal dose of barbiturate and perfused transcardially with a formaldehyde solution. The brain and vestibular nerve will be harvested for histological examination.

5. Procedural endpoint: The procedures with each animal will end approximately 90 days post-op as described above. A lethal dose of barbiturate will be administered and the animal will be perfused transcardially with a formaldehyde solution for the purpose of fixing the nerves and brain. The brain and facial nerves will be harvested for histological examination.

***Neuterina* (male cats only)**

Male cats used for survival experiments will be neutered by ULAR veterinary staff. Neutering has been recommended by ULAR veterinary staff to render the cats more behaviorally tractable so that those cats can share group housing with other cats needed for the present protocol as well as for other IACUC-approved protocols. Intact males in a colony almost certainly would fight with new intact males introduced into the colony. The neutering will take place during the period of general anesthesia needed for implantation.

Animal Monitoring

Animal Monitoring Details

Clinical Signs or Symptoms of Pain/Distress

Uncheck this box to remove all text from the box below

Terminal experiments:
Because the experiment is terminal, one of few adverse effects would be surgical pain during the procedure. When the animal is briefly allowed to awaken from the sedation, there is the possibility of pain during the 30-60 minutes of testing. As described, we will be providing the animal with appropriate analgesia for pain and discomfort control.

Survival experiments:
The most likely adverse effect would be post-surgical pain. Post-surgical pain might result in shaking, abnormal posture or walking, vocalizing, absence of grooming, and/or loss of appetite. Another possible adverse effect would be infection at the surgical and implant site. In our experience, however, such infection is a rare event. Injury to the vestibular system may cause some degree of head tilt, but this should not preclude the animal from eating, drinking, and performing activities of daily living.

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

Parameters

Terminal experiments:
The level of anesthesia will be monitored by toe pinch.

Survival experiments:
Animals will be monitored in the post-operative setting for lethargy, loss of appetite, and wound guarding. Animals will be weighed daily. The wound will be inspected daily and cleaned as needed. The behavior of the cats will be observed in the group housing setting. While in the head-fixation device, the animal can experience distress, which can be indicated by increasing heart and respiratory rate. The animal's vital signs will be continuously monitored during the course of the experiment.

Management Plan

Terminal experiments:
Animals will remain anesthetized for the duration of the experiment.

Survival experiments:
Post-surgical pain may result in shaking, abnormal posture or walking, vocalizing, absence of grooming, and/or loss of appetite, and our lab staff will be monitoring for

these signs and symptoms. To provide pain control, buprenorphine (0.01 mg/kg, s.c.) will be administered at the beginning of surgery and at 12-hr intervals thereafter for the first 48 hrs. After the first 48 hrs, buprenorphine administration will be continued if the animal shows signs of pain or distress.. Ampicillin will be given once, I.v., at the beginning of surgery. Clavamox will be given, p.o., twice daily for 14 days post-op. In the case of infection, additional antibiotics will be given with the guidance of the ULAR veterinary staff.

Frequency of Monitoring

Terminal experiments:
15-min intervals during surgery; 30-min intervals thereafter.

Survival experiments:
6-hr intervals for the first 24 hr post-op; 12-hr intervals for the next 48 hr, daily thereafter. We will work with ULAR veterinarians who will oversee post-op monitoring. Lab staff will be solely responsible for post-op monitoring and daily post-surgical care (including weekends and holidays).

Documentation of Monitoring

Terminal experiments:
A monitoring and drug-dose log will be kept during the surgery and subsequent period of data collection.

Survival experiments:
A monitoring and drug-dose log will be kept during the surgery. A monitoring chart for each animal will be kept in the animal room. The chart will list observations and treatments.

Documentation of Animal Monitoring
Lab notebook
In lab (outside of vivarium)

Endpoints

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

Experiment A

Procedural endpoint: About 4 hours after initial induction of anesthesia, data collection will be terminated and the animal euthanized. A subset of the animals will be perfused transcardially with paraformaldehyde to preserve the vestibular nerve for histological reconstruction of electrode tracks.

Experiment B

Procedural endpoint: The procedures with each animal will end approximately 90 days post-op as described above. A lethal dose of barbiturate will be administered and the animal will be perfused transcardially with a formaldehyde solution for the purpose of fixing the nerves and brain. The brain and facial nerves will be harvested for histological examination.

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

Terminal experiments:
An animal would be euthanized prematurely if physiological recording showed an irreversible decline in the animal's neurological status.

Survival experiments:
An animal would be removed from the study and euthanized if it showed intractable pain or distress, or if it developed infection that was unresponsive to antibiotics.

Euthanasia

Will animals be euthanized at the end of the experiments?
Yes
Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply
Cardiac Perfusion
What will the animals be perfused with?
It is recommended that an INJECTABLE anesthetic be used for animals undergoing cardiac perfusion
Anesthetic Overdose

Guinea Pig

Fatal Plus (a sodium pentobarbital preparation), 1-2 mL (~500 mg/kg) IP

Cat

Fatal Plus (a sodium pentobarbital preparation), 150 mg/kg IV

Confirmation of Death in Animals
Open chest inspection of the heart
You MUST...

Animal number calculation for experimental part Study Segment

Cat			
		Max	Description
		2	Experiment A, Group A
	+	8	Experiment A, Group B
	+	6	Experiment B
		16	**Cat**

Guinea Pig			
		Max	Description
		5	Experiment A, Group A
	+	30	Experiment A, Group B
		35	**Guinea Pig**

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Procedure	Species
Euthanasia followed by Tissue Harvest	Guinea Pig,Cat
Cardiac Perfusion	Guinea Pig,Cat
Induction of Anesthesia/Sedation for Non-surgical Procedures	Guinea Pig,Cat
Imaging	Guinea Pig,Cat
Post-procedural Care for Non-surgical Procedures	Guinea Pig,Cat
Pre-operative Care	Guinea Pig,Cat
Induction of Anesthesia for Surgical Procedures	Guinea Pig,Cat
Terminal Surgery	Guinea Pig,Cat
Survival Surgery	Guinea Pig,Cat
Prolonged Restraint	Guinea Pig

Procedure Descriptions

Euthanasia followed by Tissue Harvest

In selected cases, animals will be administered a lethal dose of barbiturate, followed by cardiac perfusion with paraformaldehyde (see below). The vestibular nerves will be harvested for histological examination.

Cardiac Perfusion

Animals will receive a lethal intraperitoneal dose of barbiturate. Once adequate anesthesia is achieved and confirmed with extinction of paw/limb pinch withdrawal and corneal reflexes, a thoracotomy will be performed just lateral to the sternum to expose the heart. A clamp will be placed on the descending aorta, and an 18-gauge butterfly intravenous needle will be introduced into the left ventricle. Ringer's lactate solution will be perfused through the intravenous line and into the Guinea pig and/or Cat circulation. This will be then be followed by perfusion with paraformaldehyde.

Induction of Anesthesia/Sedation for Non-surgical Procedures

Animals who underwent the survival surgery will be lightly sedated and placed in a body-restraint for video recording of eye movements by injection of acepromazine (0.1-0.15 mg/kg, i.m.). Video recording is a non-painful procedure. The purpose of the sedation and body-restraint is to minimize movement artifacts. The procedure will be begun when the animal is calm and docile within the body restraint. The animal will be attended and observed visually continuously throughout the procedure. Additional acepromazine (0.05 mg/kg) will be given if needed to suppress movements. The animal will be placed on a circulating-warm-water heating pad in the laboratory until normal mobility returns, typically

within 1 hr after the procedure.

Imaging

ISCAN (http://www.iscaninc.com/) video-based eye tracking system, or something similar, will be used to track and record the movements, specifically the direction and velocity, of the eye. This is accomplished with a simple camera and associated software.

Post-procedural Care for Non-surgical Procedures

Following a nerve stimulation session, the animal will be observed for 15-30 minutes for any residual or consequential balance issues after the stimulation. However, like any other nerve, without exogenous stimulation we do not anticipate any post-procedural balance issues.

Pre-operative Care

Animals will be housed in the ULAR-maintained vivarium and maintained according to ULAR protocol. All food will be withheld 12-18 hours prior to surgery. Water will not be restricted. In our collaborating lab's 18 years of conducting similar procedures with guinea pigs and cats, we have not observed any signs of distress resulting from this 12-18 duration of pre-op fasting. In the past, we have found that if guinea pigs and cats are fasted for only a few hours, the majority will vomit and aspirate. Early in the development of our protocol, we lost several animals to aspiration after fasting periods of only ~6 hrs. We have not lost any animals to aspiration (nor to food deprivation) since adopting the 12-18-hr fasting period. In addition, twenty to thirty minutes prior to induction of anesthesia, the animal will be given atropine via subcutaneous infection to suppress mucous secretions and dexamethasone via intramuscular injection to minimize edema and optimize function of the nerves of interest. In addition, meloxicam is given to provide analgesia during the brief period the animal is allowed to recover from anesthesia.

Induction of Anesthesia/Sedation for Surgical Procedures

INDUCTION OF ANESTHESIA

Guinea Pigs, Terminal experiments:
Anesthesia will be induced with a subcutaneous injection of a cocktail of ketamine (40-50 mg/kg) and xylazine (10 mg/kg). Supplemental doses will be given ~10 min after the initial dose until an adequate plane of anesthesia is reached. Alternatively, we may also use isoflurane via an endotracheal tube instead of ketamine and xylazine as an anesthetic in the event that recovery from ketamine and xylazine for any reason does not allow for satisfactory vestibulo-ocular reflex testing. Recovery from isoflurane is quicker and neurologic reflexes are likely repressed to a lesser degree.

Cats, Terminal experiments:
Animals will receive an initial dose of ketamine (25 mg/kg, i.m.). A 22 ga angiocath catheter will be inserted into the cephalic vein through a cut down. A surgical level of anesthesia will be induced by IV Nembutal (pentobarbital (50mg/mL), diluted 1:9 with sterile Ringers's solution, to effect.

Cats, Survival experiments:
Animals will receive an initial dose of ketamine (25mg/kg). They will be transferred to an anesthesia induction box and anesthetized with isoflurane (0.5-2% in oxygen). A 22 ga angiocath catheter will be inserted into the cephalic vein and an endotracheal tube inserted. Once in the operating room, a surgical level of anesthesia will be maintained with isoflurane in oxygen.

An adequate plane of anesthesia will be indicated by extinction of limb withdrawal in response to toe pinch.

MONITORING

Terminal experiments:
The level of anesthesia will be monitored by paw pinch at 15-min intervals during surgery. Respiration will be monitored by visual inspection and pulse oximetry. Body temperature will be continuously monitored via rectal probe. Anesthesia will be maintained with supplemental doses of ketamine (for Guinea pigs) and Nembutal (for cats). Additional xylazine may also be administered 4 to 6 hours into the procedure to maintain the surgical plane of anesthesia. We have chosen not to use artificial ventilation. The Guinea pigs and cats are not paralyzed for this procedure, and they breathe very well on their own. We have worked with this preparation for the past 6 years and have not lost any animals prior to the planned time of termination of the experiment.

Survival experiments:
Respiratory rate, EKG, oxygen saturation, end-tidal CO2, and rectal temperature will be monitored continuously, and response to toe pinch will be tested at 15-min intervals. Isoflurane levels will be adjusted to a point at which there is no limb withdrawal or acceleration of respiratory or heart rate in response to noxious stimulation. The respiratory rate of the cat will be set at15-30/min, and tidal volume at ~12 ml/kg.

Terminal Surgery

The procedure is a terminal experiment and involves implantation of a stimulating electrode array into the vestibular nerve. Twenty minutes prior to induction of anesthesia, the animal will be given atropine to suppress mucous secretions. Following induction of anesthesia, the animal will be placed on a warm-water heating pad. Body temperature will be monitored with a rectal probe and kept at ~38C. The skull will be exposed with a midline scalp incision and skin and muscles will be reflected. . In Guinea pigs, the skull will be exposed with a midline scalp incision and skin and muscles will be reflected. Lidocaine will be infused subcutaneously into the wound margins. A flat-head stainless-steel machine screw (#4-40) to be used as a head holder will be attached to the skull rostral to the coronal skull suture using stainless-steel screws and methyl methacrylate (dental) cement. Please note that the animal will be maintained in a surgical plane of anesthesia throughout the procedure, and that the head-holding device is required to hold the head securely in place to maintain head position and electrode placement during nerve stimulation.

The eyes of the animal will then be topically anesthetized with 0.2mL of proparacaine, and a plastic marker (4x4mm) will then be placed onto the cornea and/or sclera of each eye and secured with cyanoacrylate (VetBond Tissue Adhesive, 3M, St. Paul, MN). These markers allow for post-experiment analysis of the video images to determine the speed and directionality of the electrically-evoked nystagmus.

A post-auricular incision will be made to expose the tympanic bulla and occipital surface of the skull. The skull will then be opened over the lateral occiput using a carbide burr followed by Rongeurs. The final opening will be about 5-8 mm in diameter. The dura mater is removed, and the lateral one-third of the cerebellum will be excised to achieve access to the posterior surface of the temporal bone, the vestibular nerve, and the brainstem.

The penetrating electrode array has 16 iridium-plated sites, 703-µm2 in area, arrayed at 100-µm intervals spanning a distance of 1.5 mm along a single, 15-µm-thick silicon-substrate shank. The array will be advanced into position within the vestibular nerve with a

micropositioner. Stimulus presentation will use System 3 equipment from Tucker-Davis Technologies (TDT; Alachua, FL, USA) and custom software running in MATLAB (The MathWorks, Natick, MA, USA). The electrical pulses will be generated by custom optically isolated 16-channel current sources controlled by 16-bit digital-to-analog converters (TDT RX8). The current source will have a maximum output of 1 mA.

Following implantation of the array into the vestibular nerve and closure of the surgical wound, the animal anesthesia will be reversed, and the stimulation delivered to the neural implant. A consumer-grade digital video recording device will be positioned in a manner that will allow for detailed video documentation of the various movements of the eyes. The vital signs of the animal will continued to be monitored during the entire course of testing. After testing all stimulus parameters, the animal will be immediately terminated with a dose of Fatal Plus. There is no recovery period.

Survival Surgery

For the survival experiments in cats, glycopyrrolate will be given to suppress mucous secretions (0.02 mg/kg), dexamethasone (1 mg/kg) will be given to prevent cerebral edema, and buprenorphine (.01 mg/kg) will be given preemptively for analgesia. Ophthalmic ointment will be instilled in the eyes to prevent corneal drying. The animal will be placed in a standard stereotaxic frame.

For survival surgeries to implant the vestibular nerve multi-channel electrode array, a pre-auricular incision will be made with a 15-blade. Dissection will proceed to the skull, a craniotomy will be created using a diamond burr drill, until the vestibular nerve trunk is identified and skeletonized. The dissection should remain extradural; no brain tissue is removed. The nerve will then implanted with the electrode, and the electrical connections to the array will be brought to a skull–mounted connector. A stainless-steel cylinder will be placed around the skull connector to protect the connector. The floor of the cylinder and the base of the connector will be sealed with acrylic such that the inside of the cylinder is outside of biological spaces. A screw-on cap will be placed over the cylinder. The muscle and skin will be sutured closed in layers around the cylinder with absorbable sutures for subcutaneous layers and nylon suture for the skin. Triple antibiotic ointment will be applied to the wound margin. The nylon sutures will be removed after wound healing, about 7-10 days post-op. This is the established method proven effective in another IACUC approved protocol.

Surgical endpoint:
The procedures should take ~2-3 hr. Isoflurane administration will be discontinued and

oxygen delivery maintained until the cat is breathing spontaneously. The endotracheal tube will be removed.

Methods to Prevent Dehydration & Hypothermia:
During surgery, the Guinea Pigs will receive 3 cc of sterile saline subcutaneously every 2 hours. The Guinea Pigs will be held on a warm-water heating pad during the surgery.

The cats will receive ~3 cc/kg/hr Ringer's solution per hour, IV. Body temperature will be monitored using a rectal probe, and the temperature maintained at 38C using a warm-water heating pad.

Post-operative and Analgesic:
The Guinea Pigs will be euthanized following the completion of data collection.

After survival surgeries, the cat will remain on the heating pad and attended continuously until it is awake. Once awake, it will be checked at 30-min intervals until it is ambulatory and feeding, typically 2-3 hr. Then, it will be transferred to an individual cage. We will coordinate with ULAR Veterinary staff to monitor post-surgical care. Buprenorphine will be administered at the beginning of surgery and at 12-hr intervals thereafter for the 48 hrs. After the first 48 hrs. buprenorphine administration will be continued if the animal shows signs of pain or distress. The surgical wound will be inspected daily. In the absence of complications the cat will be released in the group chronic cat room as soon as the wound heals. Lab staff will be solely responsible for post-op monitoring and daily post-surgical care (including weekends and holidays).

Aseptic Techniques:
For survival surgeries, aseptic procedures will be conducted in the ULAR 3-room surgical suite. In the operating room, work surfaces will be swabbed with Sporicidin Disinfectant Solution. An aseptic area will be designated using sterile surgical drapes. For survival surgeries, all personnel will wear caps, shoe covers, masks, and gowns. The surgeon will scrub for 10 min using 2 Betadyne scrub packs, then will don a sterile gown and gloves. After induction of anesthesia, in the animal prep room, the fur on the face and scalp will be clipped, then the skin will be disinfected with alternate swabs of Betadine and alcohol (3 each). After the animal is transferred to the operating room and placed in an orbitopalatal head holder, ophthalmic ointment will be applied to the corneas, the cat will be covered with a sterile paper drape, and then an adhesive drape will be placed over the scalp. Surgical instruments will be gas sterilized and the instrument packs opened immediately prior to the surgery. Fine instruments and experimental implants will be gas sterilized. Surgical instruments will be cleaned and autoclaved between uses in multiple animals.

Neutering:
Animals will be neutered by ULAR veterinary staff at the time of general anesthesia for the vestibular nerve procedure. For neutering, the hair will be plucked over the scrotum. The skin will be disinfected with alternative scrubs with Betadine and alcohol (3 each). Ophthalmic ointment will be instilled in the eyes to prevent corneal drying. A small incision will be made through the scrotum over each testicle, the testicle will be removed, and the spermatocord and blood vessels ligated. The scrotal incision will be left open.

Prolonged Restraint

Method or Device: Screw Head Fixation

A flat-head stainless-steel machine screw (#4-40) will be attached to the skull rostral to the coronal skull suture using stainless-steel screws and methyl methacrylate (dental) cement. The machine screw threads will face away from the animal, and the screw will be secured to a rigidly fixed pole to steady the animal head.

Duration: 30-60 minutes. For these experiments, we are simply looking for stimulation of the vestibulo-ocular reflex, which would be indicated by rhythmic rapid movements of the eyes (nystagmus). Ideally, it would take less than a few minutes to achieve this and visually confirm nystagmus and to obtain approved video documentation. We ask for a longer period of time in the event there is some issue that requires adjustment or time. There will be no pre-experiment training, as this is a terminal experiment.

Monitoring: The animal will be constantly monitored during the restraint period. If the animal exhibits excessive distress, we will euthanize per protocol. The animal will be monitored for soiling and cleaned as needed.

Guinea Pig

Cat

Total number of animals

Species	Max
Cat	16
Guinea Pig	35

USDA Pain Category	Species	Number of Animals	Number of Animals
USDA Category D	Guinea Pig	35	0
USDA Category D	Cat	16	0

Animal Numbers Justification

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

Experiment A

Justification of animal numbers: Experiments consist of recording eye movements during electrical vestibular stimulation. The experimental stimulus parameters of interest include current level, pulse rate, time interval between pulses on pairs of channels, and electrode configuration (i.e., location of active and return electrodes). A pilot experiment will be conducted to work out technical challenges and to identify ranges of stimulus parameters appropriate for later systematic study. Based on the pilot results, we will identify a list of parameter values to be tested across multiple animals. The video documentation of various speeds and directions of the eye movements will provide the material needed to design a system to accurately measure electrically-evoked nystagmus. We include an additional 2 Guinea Pigs in Group B to allow for occasional failed experiments.

Our collaborator's previously published work in guinea pigs presented data from 8 to 20 animals, which does not include the approximately equal number of animals used to develop the stimulation and recording procedures and to explore the parameter spaces to establish appropriate ranges of values to test in detail quantitatively. We anticipate needing 12-17 Guinea pigs to provide usable and publishable data, and accordingly we are requesting the use of 25 to 35 guinea pigs for this protocol. Our collaborator's previously published work in cats presented data from 4-6 cats. We anticipate needing about 6 animals to provide usable and publishable data, and accordingly we are requesting the use of 8 cats for this protocol.

Experiment B

This work involves development of new surgical and testing procedures and application of new custom implants. This is challenging work, and we need to allow for the possibility of implant design modifications and some failures. In the chronic group, we have allotted up to 8 animals to allow for good quantitative data from 6 animals after a likely sizeable number of early design modifications and failures.

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

Drugs & Agents

Anesthesia

Will animals receive anesthesia agents?
Yes

Species *(receiving the drug/agent)*	Drug/Agent	Dose Range *(mg/kg body wt)*	Route *(SQ, IP, IV, IM, etc.)*	Frequency *(how often?)*	Duration *(how long?)*
Guinea pig	Ketamine	40-50 mg/kg	IM	Once, supplemented as needed	Entire course of surgery
Guinea pig	Xylazine	10 mg/kg	IM	Once	Once to induce anesthesia
Guinea pig	Proparacaine	0.2 mL	Topical (eye)	Once	After anesthesia administration
Guinea pig	Lidocaine	2 mg/kg	SC	Once	At the beginning of surgery
Guinea pig	Meloxicam	0.2 – 0.4 mg/kg	SC	Once	At the beginning of surgery
Guinea pig	Atipamezole	0.5 mg/kg	SC	Once	At the end of surgery
Guinea pig	Yohimbine	0.15 mg/kg	IM	Once	At the end of surgery
Guinea pig	Isoflurane	0.5-2% in oxygen	IH	Continuous throughout surgery	During surgery
Cat	Ketamine	25 mg/kg	IM	Once	Once
Cat	Isoflurane	0.5-2% in oxygen	IH	Continuous throughout surgery	During surgery
Cat	Nembutal	25 mg/kg	IV	~30min intervals, as needed	During surgery
Cat	Buprenorphine	0.01 mg/kg	SC	2x/day	4 days post-op
Guinea Pig	Fatal Plus (a sodium pentobarbital preparation)	1-2 mL (~500 mg/kg)	IP	Once	Once
Cat	Fatal Plus (a sodium pentobarbital preparation)	150 mg/kg	IV	Once	Once

How will the anesthesia be administered to the animals?

Will anesthetic GAS be used?
Yes

Isoflurane

Administration Method:
Uncheck this box to remove all text from the box below

Vaporizer

Waste Gas Capture Methods:
Uncheck this box to remove all text from the box below

F/Air canister

Analgesia

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

Species *(receiving the drug/agent)*	Drug/Agent	Dose Range *(mg/kg body wt)*	Route *(SQ, IP, IV, IM, etc.)*	Frequency *(how often?)*	Duration *(how long?)*
Guinea Pig	Dexamethasone	0.2mg/kg	IM	Once	Once, 30 min prior to anesthesia
Cat	Acepromazine	0.1-0.15 mg/kg	IM	Twice for sedation testing	1-2 hours each x 2 tests
Cat	Meloxicam	0.2 mg/kg	PO	Once	Once at time of surgery
Cat	Glycopyrelate	0.02 mg/kg	IM	Once	Once, 30 min pre-op
Cat	Dexamethasone	1 mg/kg IM	IM	Once	Once, 30 min pre-op
Cat	Ampicillin	10 mg/kg	IV	Once	At beginning of surgery
Cat	Clavamox	62.5 mg/kg	PO	2x/day	14 days post-op

Experimental & Other Agents

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

Species *(receiving the drug/agent)*	Drug/Agent	Dose Range *(mg/kg body wt)*	Route *(SQ, IP, IV, IM, etc.)*	Frequency *(how often?)*	Duration *(for how long?)*
Guinea Pig	Atropine	0.02 mg/kg	SC	Once	Once, 30 min prior to anesthesia

Will Controlled Substances be used?
Yes

Indicate which controlled substances will be used - Check all that apply:
Buprenorphine
Ketamine
Sodium Pentobarbital (aka. Euthasol, Nembutal, Fatal Plus)
CSUA #:
Uncheck this box to remove all text from the box below

Will non-pharmaceutical grade (i.e. chemical grade) agents be used in live animals?
No

Animal Locations & Husbandry

Food or Water Variations

Indicate the food or water variations that will be implemented in this protocol - Check all that apply:
Food Restriction (>12 hrs)
Describe if any food will be withheld from animals in order to achieve an experimental objective (e.g. motivation for behavior testing, weight control, etc.).
Uncheck this box to remove all text from the box below

All food will be withheld 12-18 hours prior to surgery. Water will not be restricted.

Justification for the Food or Water Variations
Uncheck this box to remove all text from the box below

Animals will be housed in the ULAR-maintained vivarium and maintained according to ULAR protocol. All food will be withheld 12-18 hours prior to surgery. Water will not be restricted. In our collaborating lab's 18 years of conducting similar procedures with guinea pigs and cats, we have not observed any signs of distress resulting from this 12-18 duration of pre-op fasting. In the past, we have found that if guinea pigs and cats are fasted for only a few hours, the majority will vomit and aspirate. Early in the development of our protocol, we lost several animals to aspiration after fasting periods of only ~6 hrs. We have not lost any animals to aspiration (nor to food deprivation) since adopting the 12-18-hr fasting period.

Animal Husbandry Variations

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

Justification for the Animal Husbandry Variations

Researcher-Maintained Animals

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

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

An Emergency Plan is REQUIRED for the following scenarios:

Other Husbandry/Housing Variations

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

Animal Locations

Indicate all locations where live animal procedures and/or housing will take place - Check all that apply:

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

List the lab areas (outside of the vivarium) where live animals are taken for any procedures or housing.
Uncheck this box to remove all text from the box below

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

Hazards & Safety

Chemical Hazards

Requirements for the Use of Potentially Hazardous Chemicals or Agents:
By clicking this checkbox, the PI and lab staff acknowledge that EH&S will be contacted for guidance about completing the above requirements.

Chemical Name	Hazard Type	SDS	Containment Level	Special Precautions
Paraformaldehyde	Toxin	https://www.fishersci.com/shop/msdsproxy?productName=O4042500		To be used only under the fume hood.

Biological Materials, Primary Cells or Cell Lines

Requirements for Use of Biological Materials:

Infectious Agents

Requirements for the Use of Infectious Agents:

Recombinant DNA

Preparation & Use of rDNA

Radioactive Hazards

Radiation Use Authorization (RUA) #:

Removal of Radioactive Waste & Monitoring of Radioactivity

Creation of New Transgenic Animals

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

Animal Biosafety Levels

Indicate the animal biosafety levels - Check all that apply:

Other Hazards or Safety Considerations

Other Protocol Information

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

Personnel

Principal Investigator (PI)

Faculty Sponsor

Co-Investigator/Senior Researcher

Research Personnel

Other Research Personnel

Additional Personnel Information

Emergency Contact Information

Add New User

Training Requirements

PI Qualifications

Description of PI Qualifications

Training Plan for Study Team Members

Links to Other Protocols

Other Regulatory Review Requirements

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

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

Number	Protocol Title	Document Template
		Animal Use Protocol (AUP)

PI Certification

PI Certification

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