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

Hearing-impaired individuals indicate as one of their greatest disabilities the inability to recognize or understand sounds of interest that are presented in a complex auditory background. In normal-hearing listeners, the task of picking a sound (a "signal") out of a noisy background (the "masker") is aided when the listener can distinguish the locations of the signal and masker. This involves two processes known as spatial stream segregation and spatial release from informational masking. The planned experiments will examine the brain mechanisms for those processes. We will train animals to perform spatial listening tasks similar to those performed by human listeners in coordinated perceptual studies. The animals will undergo a period of training and behavioral data collection, then we will implant chronic electrodes in the auditory cortex and record the activity of neurons while the animals are engaged in the task. We also will do similar recording using anesthetized conditions in other animals. This work has three main goals. (1) Identify the features of sound that the brain relies on for various aspects of spatial hearing. Those results will guide design of sound processors for hearing aids and cochlear implants. (2) Localize the areas of the brain responsible for those aspects of spatial hearing. Those results will aid in diagnosis and treatment of spatial-hearing disorders. (3) Characterize the influence of an animal's behavioral state on brain mechanisms of spatial hearing. Those results will give fundamental understanding of how a listener brings attention to bear on important sounds in a complex environment.

Study Characteristics

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
Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last 3 year period of this study, that was not previously reported to the IACUC?
Progress Summary
Justification for Continuing the Project
Adverse events and unanticipated problems:

There have been no adverse events.

Justification for Continuing the Project

The project is about half way through its goals of examining cortical substrates of spatial stream segregation and of spatial release from informational masking. Regarding spatial stream segregation, we have succeeded in training animals in one of two psychophysical tasks and have obtained a partial cortical data set in off-task conditions. We need to complete that data set prior to submitting a paper for peer review, and we need to collect cortical data in on-task condition. Regarding release from informational masking, we need to train animals and begin cortical data collection.

List the previously approved protocol number

Summarize the progress made on the project during the last 3-year period.

We have been successful in training cats in the stream segregation task. That work resulted in a peer-reviewed publication Efforts to record from the auditory cortex in unanesthetized animals have so far met with limited success. We have obtained valuable cortical results in animals that were awake but not engaged in a task (abstract: . That project needs data from two more animals before it can be submitted for peer review. We have not yet obtained reliable cortical recordings from animals that are engaged in a task. We have made some changes in recording-electrode design and electronics that we hope will improve data collection. That will be a major focus in the next 3-year period.

Results from this work have been presented as:

Peer-reviewed publications:

Edited books:

Abstracts:

Keynote or Other Invited Presentations:

Total Number of Animals Used in the last 3 years

10

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

Funding & Billing Information

Other Funding Sources (not captured in table above)

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

Provide information regarding peer review below if necessary.

grant eer-reviewed by and funded by

PI Home Department

Species

Cat

Cat

Species Justification

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

Cats are most appropriate for these studies because the cat has evolved accurate sound localization for its nocturnal predatory behavior, because its auditory cortex is well developed and accessible on the surface of the brain, and because the cat is capable of learning the planned psychophysical tasks. The domestic cat is well established in the literature as a model for study of the central auditory system, and a considerable amount of background information already is available for that species. A simpler animal, such as a rodent, would not be suitable because the auditory cortex is relatively primitive and because behavioral and physiological studies, at least in rats, have shown that the role of the cortex in sound localization can be fundamentally different from that in higher animals. A non-human primate might appear to be preferable to a cat because of its ability to perform complex sensory tasks and because of its closer homology to humans. Macaques are the favorites for awake-behaving studies of the visual system, but several investigators have reported great difficulty in training macaques in auditory tasks. Certain studies must eventually be conducted in primates to establish homologies with specific cortical areas in humans. Other issues, such cortical specialization for various aspects of spatial hearing, fundamentals of sensory coding by spike patterns, and effects of anesthesia and behavioral state, are readily addressable in non-primates. For that reason, there is little justification for use of primates in the present experiments.

The non-survival experiments will use both male and female cats. The survival experiments will use neutered male cats exclusively. The use of a single sex is to facilitate group housing. The choice of males is based on a recommendation from ULAR veterinary staff, in about [redacted] when the lab was being set up, that males tend to remain healthier under these husbandry conditions than females.

Animal Characteristics

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

domestic short hair cats

Phenotypic Abnormalities or Special Health Conditions

Check all that apply:
Additional information regarding the species/strain is provided below:

cats are to be purchased from an SPF colony at

Rationale & Alternatives

Search Results

Date of Search	Time period from	Time period to	Database	# of Results	Keywords
May-04-2017	Jan-01-2014	May-04-2017	PubMed	146	cat auditory cortex; auditory cortex space
May-04-2017	Jan-01-2014	May-04-2017	Web of Science	554	cat auditory cortex; auditory cortex space

Database Searches

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

The literature search did not reveal any duplicative experiments. That is, there were no reported studies that addressed our goal of examining auditory-cortical mechanism of spatial hearing that facilitate detection and recognition of sounds in the presence of other competing sounds. There are two recent studies that examined modulation of auditory cortical activity by engagement in an auditory task, but the tasks were related to tone detection or frequency discrimination, not location or stream segregation. Also, the stimulus increments to be discriminated were appreciably larger than the just noticeable difference, in contrast to our studies that will be conducted around psychophysical thresholds. There were alternative chronic cortical recording methods reported. None of those methods, however, were any less stressful to the animals than the methods that we use, and all of those procedures ultimately ended in death of the animals. For those reasons, none of the alternative methods are regarded as refinements to our 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

These experiments are part of a project that involves coordinated experiments in humans and animals. In perceptual studies in humans, we will quantify aspects of the spatial hearing capabilities of listeners and will identify the acoustic cues that they rely on. In parallel, we will explore the brain mechanisms that underlie those perceptual capabilities. Although great progress has been made in functional imaging of the human brain, no available techniques permit physiological measurements from humans at the scale that is needed for the aims of this study. For that reason, we will conduct invasive studies in animals. The animals will be trained to perform perceptual tasks similar to a subset of those done by the human listeners. After a period of behavioral testing, physiological recordings will be made from the animals' brains. For the results to be relevant to humans they must be conducted in animals in which the ear and central auditory pathway bear reasonable similarities to those of humans. Cell culture would not yield information relevant to the complex multi-level processing systems involved. Computer modeling has some value in evaluating certain functional hypotheses, and such modeling is under way in this and other laboratories, but ultimately it is necessary to look closely at the actual biological system.

Reduction

Describe the steps you have taken to reduce the number of animals to the minimum required to obtain scientifically valid data.
Answer

There have been several studies (including those from the LR's research group) that use multi-site recording arrays to increase the rate of data acquisition from each animal and, thereby, **reduce** the number of animals needed. For instance, a 32-site recording probe can increase the rate of data acquisition by a factor of 32 compared to a typical single-site tungsten electrode.

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:

The use of chronic recording system, like we propose, increases the amount of data recording time available in each animal. In each animal, we hope to collect data relevant to multiple experimental questions simply by varying the stimulus set. For instance, in an animal trained to perform a specific stream segregation task, we hope to also record off-task responses to the stimuli informational masking experiments. By **refining** our protocol to maximize data collection from each animal, we **reduce** the number of animals needed.

Study Segments

Experimental Design	Species
Acute studies	Cat
Awake recording	Cat

Acute studies

Species

Cat

Experimental Design Summary

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

Describe the rationale behind the experiment or the hypothesis being tested in this study segment.
Uncheck this box to remove all text from the box below

1. Acute Studies: Spatial hearing aids in identifying the location of a sound source (sound localization), in disentangling interleaved sequences of sounds originating at different locations (spatial stream segregation), and reducing confusion between signals of interest and other competing sounds (spatial release from informational masking). Several lines of (so-far) indirect evidence in humans and animals lead us to the hypothesis that sound localization, spatial stream segregation, and spatial release from informational masking are accomplished by different auditory cortical fields. We propose to test that hypothesis by characterizing and contrasting responses to relevant stimuli in four auditory cortical areas: the Primary Auditory Cortex (A1), the Dorsal Zone (area DZ), the Posterior Auditory Field (PAF), and the Anterior Auditory Field (AAF). Experiments consist of non-survival recordings of neural activity from the auditory cortex of cats under general anesthesia. Comparison across cortical fields will help us focus on relevant areas in the Awake Recording segment. Comparison across cortical layers should reveal the sequence of intracortical processing. Also, experiments in the anesthetized condition are needed to provide baseline data in the absence of task-dependent top-down modulation of cortical activity that we expect to see in the Awake Recording segment.

Does this study segment describe the establishment and maintenance of a breeding colony?
No

If multiple procedures will be performed under this experiment, describe the sequence and timing of all procedures for this experiment.
Uncheck this box to remove all text from the box below:

1. Non-survival neural recording from auditory cortex; Euthanasia

Animal Monitoring

Species	Monitoring Parameters	Monitoring Frequency	Responsible Person
Cat	vital signs	1/2 hr	

Animal Monitoring Details

What, if any, clinical signs or symptoms are expected in response to the procedures/manipulations in this study?
Uncheck this box to remove all text from the box below

animals remain under general anesthesia throughout each experiment

Management Plan for Animal Monitoring

Documentation of Animal Monitoring

Endpoints

Experimental Endpoints

Humane Endpoints

What are the experimental endpoints for this study segment?
Uncheck this box to remove all text from the box below

In these non-survival experiments, data collection will continue as long as cortical responses to sound appear normal, up to ~48 hr. The goal of this lengthly period of data collection is to maximize the data collection from each animal, thereby minimize the number of animals needed.

What clinical signs or other criteria will be used to determine that an animal must be removed from the study ahead of schedule (Humane Endpoint)?
Provide additional details about any checked items, or any other endpoint criteria:

Data collection will be terminated prior to the target of ~48 hours if neural waveforms diminish markedly or become unresponsive to sound stimulation. Also, data collection would be terminated in the event of some intractable problem in maintaining the intended level of anesthesia.

Euthanasia

Will all research animals be euthanized at the conclusion of this experiment/study segment?
Yes

Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply
You MUST...

Animal number calculation for experimental part Acute studies

Cat		
	Max	Description
	16	1
	16	**Cat**

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Procedure	Species
Neutering	Cat
Non-survival neural recording from auditory cortex.	Cat
Psychophysical training and testing	Cat
Implantation of chronic recording array	Cat
Euthanasia	Cat

Procedure Descriptions

Neutering: All of the animals use in the awake-recording studies will be neutered by ULAR veterinary staff for the purpose of reducing aggressive behavior, thereby making it possible to introduce new animals to the housing group. 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. All cats will receive a single dose of meloxicam at 0.2 mk/kg i.m.

For females, the hair on the ventral abdomen will be clipped and 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. An incision (2-3 cm in length just cranial to the umbilicus) will be made in the ventral abdomen. The uterus and both ovaries will be located and removed; vessels to the ovaries and the uterine stump just proximal to the cervix will be tied off with 2-0 vicryl (or similar suture). The abdominal wall will be sutured with 2-0 vicryl (or similar suture) and the skin will be sutured with 2-0 silk (or similar suture). All cats will receive a single dose of meloxicam at 0.2 mg/kg IM or Simbadol, with additional doses, as needed. Skin sutures will be removed 10-14 days post-surgery.

Non-survival neural recording from auditory cortex. General anesthesia will be induced using an i.m injection of ketamine (25 mg/kg) and acepromazine (0.1 mg/kg) followed by administration of Isoflurane (0.5-2.0% in oxygen) through a commercial anesthesia mask. An endotracheal tube will be inserted through a tracheostomy for continued Isoflurane administration. A catheter will be inserted in a forelimb vein through a cut-down. A surgical level of anesthesia will be maintained throughout the ~2-hr surgical procedure. During and ~1 hr after the end of surgery, we will gradually transfer the animal from isoflurane to alpha-chlorolose anesthesia and will maintain the animal on alpha-chlorolose throughout the ~2-day data-collection period. Alpha-chlorolose is favored for these auditory cortex experiments because it tends to produce an acceptable level of anesthesia while leaving the cortex relatively active. We have used this anesthesia protocol successfully for the past [redacted] yrs. Alpha-chloralose is not available in a pharmaceutical grade. We use Reagent grade. The powder is dissolved in pharmaceutical grade propylene glycol using a sterile beaker and stir bar to make a stock solution of 25 mg/ml. The solution is filtered with a sterile syringe filter (0.22 µm pores) into a sterile vial with a silicone septum. Loading doses are given using the stock solution, typically 3-4 injections of 0.5 to 2 ml, 25 mg/ml, i.v., a total of ~45 mg/kg). A dilute solution for maintenance doses is made by adding 32 ml of the stock solution to a sterile 500-ml bag of Ringer's solution. An i.v. drip of lactated Ringer's solution containing alpha-chloralose will be begun, at a rate of 6-12 drips per minute. The dorso-lateral surface of the skull will be exposed by a midline scalp incision and reflection of the temporalis muscle. A stainless steel fixture will be secured to the skull with screws and methacrylic cement. The fixture will be used to support the cat's head in the sound booth. The auditory cortex will be exposed by opening a ~1-cm-diameter hole in the skull using a cutting burr and Rongeurs. The dura mater will be left intact at this point. Wound margins will be infused with bupivicane, s.c. After completion of the skull opening and ~2 hr of alpha–chloralose infusion, the gas anesthesia will be discontinued and anesthesia maintained with alpha–chloralose alone throughout the duration of data collection. Oxygen delivery will continue through the tracheostomy tube using a rebreather circuit. After discontinuation of the Isoflurane, the animal will be transferred to a sound-treated booth, with its body supported by a circulating-warm-water blanket in a sling and its

head supported by a bar attached to the stainless-steel skull fixture.Data collection is carried on continuously, 24 hrs per day for a total of ~48 hr, and involves presentation of acoustical stimuli and neurophysiological recording of brain activity. The animal is attended continuously by one or more personnel working in shifts. During data collection, heart rate and body temperature will be monitored continuously and logged at 30-min intervals. Lactated Ringer's solution will be administered i.v. by a continuous drip (3 cc/kg/hr). The 2–day duration of data collection is needed to accommodate the lengthy stimulus set that must be tested at multiple electrode sites. Also, lengthy experiments maximize the data yield from each animal, thereby reducing the number of animals that are needed. Neurophysiological recordings are made using 32-site silicon-substrate recording probes, each probe 15 µm thick and 150 µm in width tapering to 15 µm near the tip. A micromanipulator is used to insert the probes through small punctures in the dura mater. Typically, we record from 2 probes simultaneously. After the insertion of the probes, the brain surface is covered with warmed 4% agarose in water, which cools to form a gel coating over the brain, thereby maintaining reducing brain pulsation and preventing drying of the cortical surface.At the end of ~2 days of data collection, or if there is an indication of a decline in cortical responses, the animal will be euthanized with an i.v. injection of an approved barbiturate-based euthanasia solution (e.g., Fatal Plus, 150 mg/kg).

Psychophysical training and testing: Animals will be trained in a psychophysical (i.e., quantitative perceptual) task to evaluate the spatial acuity of SSS and SRIM. The psychophysical training and testing are of value on their own for the purpose of comparing the cat animal model with human listeners indeed, we have already published basic SSS psychophysical results from one block of animals [redacted]. Animals trained in the SSS or SRIM task also are needed for the subsequent awake recording. In both SSS and SRIM tasks, the cat begins each trial by depressing and holding a pedal, which will initiate a sequence of background sounds. After a variable time period, a target sound will appear. The target is a change in rhythm of a pulse rate for SSS and an onset of a constant-frequency sequence of tone pips for SRIM. The cat will be rewarded for releasing the pedal within 1800 ms of the appearance of the targe. The reward will be a small portion of pureed canned cat food. Early releases or misses will be punished with a 4-second time out period. As is standard practice in UCI ULAR husbandry, cats will receive food once per day. In this case, the food will consist of that received as rewards during the psychophysical procedure. In addition, cats will be given free access to dry food for a limited period after each daily testing session. Cats will be lightly restrained during each testing session. The restraint consists of a commercial restraint bag attached to the testing platform in a way that will permit free standing or sitting and movement of the head and limbs but which will prevent the animal from jumping off the platform or turning more than about 180°.

Implantation of chronic recording array. A minimum of 6 months will pass between the spay or neuter procedure and the array implantation. For the implantation, dexamethasone (1 mg/kg) will be given to prevent cerebral edema, and buprenorphine (0.01 mg/kg) will be given preemptively for analgesia. Ophthalmic ointment will be instilled in the eyes to prevent corneal drying. The animal's head will be supported in an orbito-palatal head holder.A midline scalp incision will be made through the adhesive drape. Superficial muscle and fascia will be cleared to expose the dorsal skull surface. The temporalis muscle on one side will be reflected, and a ~1-cm-diameter opening will be made over the auditory cortex. Then the chronic recording array will be implanted based on surface landmarks using a micromanipulator. The array will consist of 2-4 shanks, each having 8-16 recording sites. The sites on the array will connect with a flexible cable terminating in a percutaneous connector. After the insertion of the array, the cable will be led medially and the connector mounted near the midline of the skull. Exposed brain surfaces will be covered with silastic (Kwil-Sil, World Precision Instruments) and then with methacrylate cement anchored with stainless-steel bone screws. In most animals, a second implant will be placed in auditory cortex in the contralateral hemisphere and a connector brought to the midline. A stainless-steel cylinder (2.5-cm diameter) will be placed over the two connectors. The cylinder will be secured with methacrylate cement anchored with bone screws. The floor of the cylinder will be covered with methacrylate, sealing the inside of the cylinder from biological spaces. The purpose of the cylinder is to protect the connectors from mechanical damage. A screw-on cap on the cylinder will be maintained except during recording procedures The skin will be sutured closed in layers around the cylinder. Absorbable discontinuous sutures will be used for subcutaneous sutures, and 4-0 nylon running sutures will be used 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.

Euthanasia: Injection of a commercial barbiturate-based euthanasia agent (e.g., Fatal Plus, 150 mg/kg). In the case of the acute recordings, in which the animal is already anesthetized and in which an i.v. line is available, the injection will be given i.v. In the awake-recording animals, the animal will first be sedated with ketamine (25 mg/kg) and acepromazine (0.15 mg/kg) followed by an i.p. injection of the euthanasia agent.

Cat

Awake recording

Species

Cat

Experimental Design Summary

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

Describe the rationale behind the experiment or the hypothesis being tested in this study segment.
Uncheck this box to remove all text from the box below

2. Awake Recording: We aim to understand the cortical mechanisms that help listeners understand sounds in complex acoustic background, such as a busy office, restaurant, or classroom. In human subjects, we are characterizing two relevant aspects of spatial hearing: spatial stream segregation (SSS) and spatial release from informational masking (SRIM). We plan to train cats to perform hearing tasks similar to those used on our ongoing human psychophysical studies, then to record from neurons in the auditory cortex while the cats perform the tasks. The general goals are as follows: (1) to quantify each cat's accuracy in one of two listening tasks (2) to study cortical responses to the auditory stimuli presented during those tasks to identify features of neural responses (e.g., spike counts or spike timing) that show stimulus dependence similar to that of the behavior; (3) to compare cortical responses across cortical layers to explore intra-cortical processing mechanisms; (4) to compare responses among auditory cortical fields to identify fields that might be specialized for particular aspects of spatial hearing; and (5) to monitor the influence of the animal's on- or off-task behavioral state on cortical responses. Each animal will be trained on only one of the two tasks, but whenever possible, pilot off-task cortical recordings will be obtained from the non-trained task at the conclusion of recording sessions.

2.A. Spatial Stream Segregation (SSS): The stimuli are sequences of broadband noise bursts interleaved in time from two (source and masker) locations. The animal will be trained to detect a change in the rhythm of the source. Training in the SSS will be completed in 3 animals that currently are in training, and training will be begun in an additional 11 animals. After attainment of an asymptotic level of performance (~4-6 months), each animal will be implanted with a 32-channel recording electrode array. Following recovery from the surgery, daily awake recording will be begun, continuing for ~6 months or as long as the animal remains healthy and cortical activity can be recorded.

2.B. Spatial Release from Informational Masking (SRIM): The stimuli are complexes of tone pips, varying in frequency. The animal will be trained to detect the onset of signal consisting of a constant-frequency series of pips. Animal training and awake recording for this segment are identical to those in segment 2.A. except for the difference in stimulus set. This group will consist of 15 animals.

Does this study segment describe the establishment and maintenance of a breeding colony?
No

If multiple procedures will be performed under this experiment, describe the sequence and timing of all procedures for this experiment.
Uncheck this box to remove all text from the box below:

2.A. Neutering; Psychophysical training and testing; Cortical recording in behaving animals; Euthanasia

2.B. Neutering; Psychophysical training and testing; Cortical recording in behaving animals; Euthanasia

Animal Monitoring

Species	Monitoring Parameters	Monitoring Frequency	Responsible Person
Cat	signs of distress	daily	
Cat	signs of distress	daily	ULAR personnel

Animal Monitoring Details

What, if any, clinical signs or symptoms are expected in response to the procedures/manipulations in this study?
Uncheck this box to remove all text from the box below

none

Management Plan for Animal Monitoring

Documentation of Animal Monitoring

Endpoints

Experimental Endpoints

Humane Endpoints

What are the experimental endpoints for this study segment?
Uncheck this box to remove all text from the box below

Experiments will continue for about 6 months after recovery from the implantation of the recording device.

What clinical signs or other criteria will be used to determine that an animal must be removed from the study ahead of schedule (Humane Endpoint)?
Provide additional details about any checked items, or any other endpoint criteria:

Signs of intractable pain or discomfort or infection

Euthanasia

Will all research animals be euthanized at the conclusion of this experiment/study segment?
Yes

Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply
You MUST...

Animal number calculation for experimental part Awake recording

Cat		
	Max	Description
	25	1
	25	**Cat**

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Procedure Descriptions

Cat

Total number of animals

Species	Max
Cat	41

USDA Pain Category	Species	Number of Animals	Number of Animals
USDA Category D	Cat	41	0
			0

Animal Numbers Justification

Explain how the animal numbers were determined.

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

Provide the scientific justification for the number of animals required for these studies.
Uncheck this box to remove all text from the box below

1. Acute Studies. Experiments consist of non-survival recordings of neural activity from the auditory cortex of cats under general anesthesia. We record using 32-site recording probes oriented across the thickness of the cortex (i.e., sampling multiple layers simultaneously). We plan to characterize responses in 4 auditory cortical fields, using sound stimuli intended to discriminate localization, stream segregation, and informational masking functions. We need to collect data from ~20 probe placements in each of areas A1, DZ, PAF, and AAF to obtain the needed sample of frequency tuning and binaural specificity, a total of ~80 probe placements. Typically, we record with 2 32-site probes simultaneously, and generally can study 3 or more pairs of probe placements in each animal (i.e., 6 placements per cat). Therefore, we estimate that we need complete data from ~14 or more cats. We have added 2 to the animal count to allow for some pilot studies and to allow for occasional failed preparations. That total of 16 cats is an outside estimate. We hope to reduce the animal use by extending each experiment over several days to maximize the data that can be collected from each animal.

2. Awake recording. For each of the two groups (i.e., 2.A. SSS and 2.B. SRIM), we plan to compare responses in cortical area A1 with responses in 1 or 2 other cortical areas. We estimate that that will require a total of 20 successful probe placements for each of the 2 groups to provide some indication of between-site variance and a sample of low- and high-frequency-sensitive sites in each field. We will attempt to place one 32-site recording probe in each hemisphere of each cat, but we cannot count on successful recording from each probe. We estimate a need of 14 cats in each group to obtain 20 successful placements in each group, a total of 28 cats (25 new cats plus 3 that are already in training).

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

Drugs & Agents

Anesthesia

Will animals receive anesthesia agents?
Yes

Species	Name of Drug/Agent	Dose Range *(list dose in mg/kg body weight)*	Route *(SQ, IP, IV etc.)*	Frequency *(how often given?)*	Duration of Treatment *(how long will treatment last?)*
Cat	Ketamine	25 mg/kg	IM	Once	Once
Cat	Acepromazine	0.1 to 0.15 mg/kg	IM	Once	Once
Cat	Isoflurane	0.5 to 2.0% in oxygen	IH	continuous during surgery	duration of surgery
Cat	Cetacaine	1 1-second spray	spray	once during tracheal intubation	once
cat	alpha-chloralose	45 mg/kg loading dose	IV	once	once
cat	alpha-chloralose	~20mg/kg/hr	IV	continuous drip	through 24-hr acute experiment

How will the anesthesia be administered to the animals?

Will anesthetic gases be used in this project?
Yes

Method of Administration:
Answer:

vaporizer, 0.5-2.0% in oxygen

Agent Used:
Answer:

isoflurane

Method Used to Capture Waste Gases:
Answer:

re-breather circuit with F/Air

Analgesia

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

Species	Name of Drug/Agent	Dose Range *(list dose in mg/kg body weight)*	Route *(SQ, IP, IV etc.)*	Frequency *(how often given?)*	Duration of Treatment *(how long will treatment last?)*
Cat	buprenorphine	0.005-0.01 mg/kg	SC	12-hr	2 days
cat	Meloxicam or Simbadol	0.2 mg/kg	IM	once	Pre-op (additional tx as needed)
cat	dexamethasone	1 mg/kg	IM	once	pre-op
cat	ampicillin	10 mg/kg	IV	once	pre-op
cat	clavamox	62.5 mg/kg	PO	2/day	14 days

Experimental & Other Agents

Will other agents be administered to animals?
No

Will Controlled Substances (as defined by the U.S. Drug Enforcement Administration) be used in the study?
Yes - list CSUA number:

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

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

Provide justification for the use of non-pharmaceutical grade drugs in live animals.
Describe how the drug will be prepared before being given to the animals.
Describe how the drug will be prepared for administration in live animals (e.g., reconstitution, filtering, etc.)

Alpha-chloralose is not available in a pharmaceutical grade. We use Reagent grade. The powder is dissolved in pharmaceutical grade propylene glycol using a sterile beaker and stir bar to make a stock solution of 25 mg/ml. The solution is filtered with a sterile syringe filter (0.22 µm pores) into a sterile vial with a silicone septum. Loading doses are given using the stock solution. A dilute solution for maintenance doses is made by adding 32 ml of the stock solution to a sterile 500-ml bag of Ringer's solution.

Is a pharmaceutical-grade equivalent available for use?
No

Animal Locations & Husbandry

Food or Water Variations

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

Justification for the Food or Water Variations

Do the experiments or procedures require any special dietary requirements or restrictions, or additions to the normal drinking water?
No

Will food or fluid be restricted on this protocol?
Yes

Describe in detail the food or water restriction procedures.

During periods of psychophysical data collection (normally 5 days per week), cats will receive all their food in the laboratory, once a day. They will receive canned commercial cat food as behavioral reinforcement during psychophysical trials. These trials last as long as the cat is willing to work, typically 30-60 min. After trial completion, the cat will be offered ad libitum dry cat food for ~30 min prior to return to the vivarium.

Animal Husbandry Variations

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

Justification for the Animal Husbandry Variations

Will there be deviations from standard environmental enrichment on this protocol?
No

Will wire-bottom caging be used?
No

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:

Will animals be held outside of ULAR animal facilities (e.g. in the researcher's laboratory) for more than 12 hours?
Yes

Location:
Answer:

Acute (non-survival) experiments last ~48 hr in laboratory

Scientific justification for housing animals in the laboratory:
Answer:

48 hr hours is the time needed to accomplish the experimental goals.

Describe how animals will be monitored and what care they will receive while in the lab:
Answer:

vital signs are monitored at 30-min intervals

Will animals be held outside of ULAR-controlled animal facilities for more than 24 hours?
Yes

Preferred light cycle:
Answer:

Animals are anesthetized throughout the 48-hr recording phase of these experiments. Recording conditions are dark.

Preferred room temperature:
Answer:

animal's core temperature is monitored with a rectal probe and maintained with a warm-water heating pad

Cleaning of Primary Enclosures:
Answer:

cleaned before and after the experiment

Feeding regimen:
Answer:

IV drip with 5% dextrose in lactated Ringer's solution

Will animals be held outside of ULAR-maintained vivarium space for more than 7 days?
No

Will the research team provide routine husbandry and care for animals housed in ULAR animal facilities?
No

Other Husbandry/Housing Variations

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

Will there be other husbandry deviations or special considerations regarding the animal use in this protocol?
No

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?

Will any non-UCI site(s) and/or offsite locations be used for any procedures on UCI-owned animals?
No

Hazards & Safety

Chemical Hazards

For each chemical hazard listed above, you must (check items once they have been completed):
Submit a Standard Operating Procedure (SOP) for safe handling and disposal of contaminated materials.

Chemical Name	Hazard Type	SDS	Containment Level	Special Precautions
alpha chloralose	acute toxicity, oral inhalation, or skin	attached		This agent is dissolved, 1 g in 40 ml propylene glycol. Solution is prepared in a chemical fume hood. Technician wears gloves, goggles, and a mask. Solution is administered to animal through an i.v. line.

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
Emergency Contact:

Add New User
Research Personnel

Name	UCInetID	UCI Email

Training Requirements

PI Qualifications

Description of PI Qualifications

Training Plan for Study Team Members

Describe the Principal Investigator's Qualifications to perform or oversee the research.
Uncheck this box to remove all text from the box below

has a PhD in from and post-doctoral training at has more than years experience in using cats.

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.

Does this research involve the use of human adult or embryonic stem cells (including induced pluripotent stem cells) in live animals?
No

Is Institutional Biosafety Committee review/approval needed for any part of this research?
No

Is Institutional Review Board (IRB) review/approval needed for any part of this research?
No

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.