Summary	
Principal Investigator :	Name Redacted, Redaction Code: F6037
Title :	Effects of Learning on Speech Representation in Auditory Cortex
Purpose :	Research
Objectives :	The purpose of this research is to help understand how the auditory cortex processes complex sounds in an animal model where the organization of auditory cortex is known to closely resemble that of humans. Such a model provides access to the cellular basis of auditory processes underlying the perception of phonetic features and is crucial for the development of therapeutic approaches to speech perception disorders such as in dyslexia, hearing loss, and aphasias. The functional organization of the cortex refers to the way that the tuning of individual neurons for different aspects of sounds (e.g., frequency and intensity, corresponding to percepts of 'pitch' and 'loudness') is distributed in the brain. Although we know that tuning of neurons in the primary auditory cortex (AI) of mammals is organized by a number of simple sound features (e.g., frequency) when tested with simple signals such as pure tones, much less is known about how sensitivity to more complex sound features (e.g., more complicated distributions of sound energy across multiple frequencies, or patterns of intensity changes over time) is organized in AI. A better understanding of these organizational principles is necessary in order to understand the processing of complex signals such as human speech. This research will use a range of mathematical techniques to develop computational models of the response properties of cortical neurons, and compare those models to the responses of actual cortical neurons to complex sounds that share important features of speech (e.g., monkey vocalizations), or are presented in environmental conditions that mimic those that cause problems for human listeners (e.g., background noise). Towards this end, we will be conducting experiments in normal animals and animals that have been partially deafened unilaterally. These experiments are described in Section G of this protocol. The overall goal is to determine central auditory deficits following partial hearing loss and to develop potential therapeutic strategies for humans. The use of awake nonhuman primates for this work is essential for two reasons. First, anesthesia has much larger effects on the responses of cortical neurons than it does on the responses of neurons earlier in the auditory pathway, such as the brainstem. The effects of anesthesia are also most likely to perturb responses to sounds, like speech, that exhibit dynamic changes in both frequency and intensity. Second, physiological research has demonstrated that the organization of auditory cortex for simple sounds is much more similar between humans and nonhuman primates (including squirrel monkeys), than between humans and other common animal models, such as cats and rodents.
Status :	Approved
Expiration Date :	03/06/2021
	Approving Veterinarian's Name Redacted

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Application History			
Project Number	**Approval Type**	**Approval Date**	**Expiration Date**
[redacted]	Modification		03/06/2021
[redacted]	Modification	03/23/2020	03/06/2021
[redacted]	Annual Review/Modify	03/06/2020	03/06/2021
[redacted]	Modification	11/06/2019	03/06/2020
[redacted]	Modification	10/14/2019	03/06/2020
[redacted]	Modification	07/23/2019	03/06/2020
[redacted]	Annual Review	02/05/2019	03/06/2020
[redacted]	Modification	11/27/2018	03/06/2019
[redacted]	Modification	10/30/2018	03/06/2019
[redacted]	Modification	07/11/2018	03/06/2019
[redacted]	Continuation	03/06/2018	03/06/2019

Modification Description and Rationale

Are these Administrative Changes Only?	Yes

Provide a brief description of the protocol changes and the REASON(s) for these changes. Indicate each section of the application that you are modifying.

Adding [redacted] to section D.

A. Funding

A.1
Type:
Federal, State or Other Government

A.2		
Funding Agency/Sponsor	**Grant or Contract Number**	**Source has Approved Method for Scientific Merit Review**
NIH/NIDCD	Redacted	Yes
NIH/NIDCD	Redacted	Yes

You have selected at least one Project Funding Sources with an IACUC approved Method for Scientific Merit Review.

B. Regulated Materials and Stem Cell Info

B.1			
Biological Materials	[redacted]		**09/01/2020**
	[redacted]		09/01/2020
Controlled Substance	[redacted]	2016-CS-01	10/14/2018
	[redacted]	2016-CS-01	10/14/2018
	[redacted]	2016-CS-01	10/05/2020

B.2

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Will data from this study be used to apply for Food and Drug Administration (FDA) approval of a drug or device, or will animal studies be outsourced?
No

B.3	
Use of Human Embryonic Stem Cells:	
Will human embryonic stem cells or human somatic stem cells be used under this protocol?	No
Are the human embryonic stem cells on the NIH Human Embryonic Stem Cell Registry?	No
Does this protocol involve the transfer of a human somatic cell nucleus into an animal egg?	No
Does this protocol involve the combination of human embryonic stem cells with an animal embryo?	No

C. Animals

C.1						
Species	**Age**	**Weight Range (kg)**	**USDA Pain Category**	**Acquired**	**Bred**	**Total**
Monkey, Squirrel	Adult		D	21	0	21
			Total:	21	0	21

C.2

As required by federal regulations, describe the statistical tests (e.g. Power analyses) and/or other rationales (e.g. Tissue collection needs, breeding efficiency) that you used to determine the number of animals requested above. Note: The IACUC may require that you consult with a statistician from the UCSF Division of Biostatistics (476-8671).

We perform two types of experiments: acute experiments in anesthetized, untrained animals, and chronic recordings in awake animals, usually followed by acute anesthetized recordings in the chronic animals. We have one project (speech representation in monkey auditory cortex), supported by the NIH, requiring 3 animals per year, and one project (hearing loss) that, by itself, requires 4 animals per year (3 experimental, one control) for a total of 7 animals. However, some of the experiments proposed in the speech project can be obtained from the same animals used for the hearing loss project. We will attempt to reduce the total number of animals by collecting data for both projects whenever possible.

We aim to collect data from a minimum of 3 animals for each scientific aim in the chronic/awake recording projects. This number does not reflect the outcome of a statistical analysis of animals as separate experimental units; instead it alleviates the concern that the results of our statistical tests, based on the number of studied neurons, are not idiosyncratic to a single animal, for example due to differences in prior experience to sounds or due to differences in the induced hearing loss. By replicating findings in 3 animals (on two hemispheres each), this concern is alleviated. In addition, due to technical factors (like excessive build-up of granulation tissue or instability of the recording cylinder) recordings are unsuccessful or unobtainable in about a quarter of hemispheres. The proposed number of animals is the minimum to address all of these scientific and technical issues.

Data from each experiment will be analyzed with a variety of statistical techniques, although we favor non-parametric resampling techniques such as Permutation Tests (Good, Permutation Tests, Springer, 2000) and Bootstrap Analyses (Efron and Tibshirani, An Introduction to the Bootstrap, Chapman and Hall, 1993), since these types of data are typically not normally distributed.

The number of neurons required to address our scientific questions ranges in quantity, but a minimum of at least 300 neurons (corresponding to 20-30 multi-electrode array penetrations) is required to establish statistical

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significance, based on $\alpha = 0.05$, and a goal of detecting a 7.5% difference in temporal or firing rate responses with a power of 0.80.

Partial Hearing Loss animals: Each study in non-human primates requires a minimum of three experimental animals to prove that the observed effects in one animal generalize across minor inter-individual differences. Therefore we require initially three sufficiently treated/exposed animals and one control animal as an electrophysiological and histological reference. The success rate of inducing the desired mild to moderate sensory-neural hearing loss (SNHL) has been high in our experience (we have treated so far more than 10 animals this way).

C.3
Will you be performing animal imaging or other animal research procedures using equipment or facilities used by human patients or human subjects?
N/A

C.4
Will you be using any animals transferred from another PI or protocol, from your previous protocol, or transferred from another Investigator or Institution?
Yes

C.5
Will animals have undergone any procedures?
Yes

C.6
Describe the prior experimental procedures, justify the use of the animals for your research and submit a completed LARC Animal Transfer Form with your application or at the time of animal transfer.
Two animals from our prior protocol have already undergone surgery for headrest attachment and recording cylinder placement.

C.7
Justify also if the animal(s) is/are used in more than one protocol involving a major operative procedure from which it is allowed to recover.
The animals are on the same project but the project spans differently numbered (but the same) IACUC protocols. There is only ONE active protocol.

D. Contacts & Personnel

Redact Code / *Role(s)	Degree	Title
DILN		POSTDOC-EMPLOYEE
PIB *Alternate Responsible	MS	SRA 3
6JCA	PHD	POSTDOC-FELLOW
E6023	MD	PROF-HCOMP

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B0OD	PHD	POSTDOC-FELLOW
BJJH		Student
32H2		
3BO0 *Alternate Responsible *Authorized Purchaser *Correspondence To *Emergency Contact 2	PHD	ASST ADJ PROF-HCOMP
F6037 *Principal Investigator *Emergency Contact 1	MD, PHD	PROF-HCOMP

E. Justifications and Alternatives

E.1.1

Sources

Date of Most Recent Search	Key Words	Search Site	Years Covered
2/3/2020	auditory cortex, monkey, squirrel monkey, non-human primate, analgesia, fluid/food restriction, alternatives to water restriction, scheduled feeding, positive reinforcement training techniques, instrumental learning, classical conditioning, behavioral training technique, vascular perfusion, craniotomy, chronic recordings, behavior, surgery	Google scholar, Pubmed	1967-2020
2/3/2020	auditory cortex, monkey, squirrel monkey, non-human primate, analgesia, fluid/food restriction, alternatives to water restriction, scheduled feeding, positive reinforcement training techniques, instrumental learning, classical conditioning, behavioral training technique, vascular perfusion, craniotomy, chronic recordings, behavior, surgery	ISI Web of Knowledge	1970-2020

E.1.2

Other Resources

Date	Topics	Resource (e.g. Attendance at meetings, consultation w/ colleagues)
1/29/2020	Auditory Neuroscience	Annual meeting Association for Research in Otolaryngology
10/23/2019	Neuroscience	Society for Neuroscience annual meeting

E.2.1

Explain why animals are required for your studies, and why replacements, such as cell culture or computer modeling, cannot fully replace animals.

We are studying learning-induced changes in the brain in response to auditory signals. These studies require organs of hearing, an intact brain, and a model that changes behavior and neuronal encoding in response to auditory cues. At present,

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not enough is known about these interactions to develop a computer model. We hope, however, that data from the proposed studies will help to develop computational models in the future.

Further, there is, at the present time, no non-invasive way in animals or humans capable of providing the level of detailed information about distributed auditory neuronal coding and cell assembly formation that is required for understanding the fundamental underlying mechanisms of learning in the auditory cortex. Current functional imaging techniques are still too coarse to resolve spatial location and timing of action potentials at the level of single neurons. Thus, information about auditory learning in the cerebral cortex can only be defined by studies that employ microelectrode response mapping studies on intact, behaving animals.

E.2.2

Explain why the proposed species are the most appropriate.

Squirrel monkeys are chosen for this study because: 1) the auditory system is more closely related to humans than that of cats or rodents. 2) Core auditory cortex in New-world monkeys is located largely at the surface of the brain and easily accessible for electro-physiological recordings unlike for NHPs such as macaque monkeys where it is located deep within the brain. Auditory cortex in squirrel monkeys is spatially organized for several spectral and temporal response parameters, which permit structure and function correlative studies, 3) Previous studies in this species, including from our laboratory, are available, which provide background and context for the studies in this protocol. 4) Squirrel monkeys are often bred in captivity for research, so their use poses no threat to wild populations.

Furthermore, the squirrel monkey, in contrast to cats, can perform behaviors at rates comparable to humans while under passive restraint that makes neural recordings possible. Squirrel monkey can perform behaviorally for up to several hundred rewards per training session. This will allow us to drive behavioral plasticity changes at a higher rate and with more consistent success than in cats or rodents. It will allow us to record large amounts of data from a small number of behaving animals and with reference to the internal cortical organizations.

Other non-primate species have a comparatively poor record at achieving the levels of performance in the types of behaviors most commonly used to evaluate human auditory responses. Furthermore, recordings while under passive restraint in non-primate species are primitive and limited in scope compared to what may be achieved with the squirrel monkey. Combined behavioral and electrophysiological experiments in macaque monkeys is possible but with a significant reduction in spatial resolution and yield due to the unfavorable location of auditory cortex for electrode placement.

E.2.3

Describe how your proposal minimizes animal pain and distress (e.g. use of in vitro procedures, reduction of animal numbers, refinement of experimental design, refinement of procedural techniques). Please be specific.

REDUCTION OF ANIMAL NUMBERS:

Efforts to minimize the number of animals include: 1) use a longitudinal model in which changes in the physiological state of the cortex are studied. In this way each animal serves as its own control, 2) optimal execution of behavioral training and brain mapping procedures produce a large body of information within single animals with a minimum of damage to the cortex, thereby reducing variance associated with pooled data across subjects; this enables a reduction in the number of animals necessary to show a statistically significant effect, and 3) continuous statistical analysis of experimental data to achieve target statistical resolution with a minimum number of animals.

By continuously analyzing experimental data as it is being collected, we can ensure that when the minimal data requirements for a given project are met, that animal can be transitioned to another experiment (to maximize yield per animal), or taken off study (to minimize stress). In studies where the animal is simply listening passively to different stimuli, changing the experiment entails changing the stimuli presented to the animal. From the perspective of animal welfare, there is no meaningful change when this is done. From the perspective of achieving our scientific goals, however, we will have reduced the total number of animals needed to complete a set of studies.

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For research projects involving neurophysiology, the relevant "N" is the number of recorded cells, not the number of animals. As a result, bias is not a concern in the way it would be for studies where animals are grouped (experimental group, control group, etc.), and halting the study after the results have been analyzed (and the desired result obtained) would be problematic. The analyses we will perform on a continuous basis, in this context, would be done simply to ensure that an adequate statistical sample has been achieved (for example, neurons tuned across a representative range of frequencies). That is, we would be using the continuous analyses only to confirm that a statistically adequate sample had been obtained (e.g., neurons spanning the cortical laminae had been recorded), not that a desired result had been observed (e.g., laminar differences in a physiological property of interest).

We continually survey the literature published in our research field. We as a group subscribe to the major scientific journals that publish most reports in our subdiscipline; we attend all major scientific meetings in our research area; several of us travel extensively and sustain direct communication with scientists conducting research that is most directly related to our own. Through this complex process, we are assured that our studies are not duplicating experiments conducted earlier or in parallel by other scientists in our field. At this time, there is inadequate information about functional and anatomical constructs of the auditory cortex to implement a valid computer model. However, one of our experimental objectives is to develop the basis for computational models. This parallel computer modeling approach is contributing to our understanding of brain mechanisms underlying neurobehavioral mechanisms and contributes progressively to the minimization of the numbers of animal studies.

RELIEF METHOD:
Chronic recording animals adapt quickly to these experimental conditions. Animals seldom show signs of discomfort with the skull implant after a few days following surgery. Recording procedures involve minimal discomfort. For short procedures that cause more than slight or momentary discomfort, local anesthesia (1% lidocaine) and/or ketamine or ketamine and Midazolam will be used. For major surgical procedures Isoflurane or Sevoflurane gas inhalation will be administered. Analgesic drugs will be used to treat peri- and post-operative pain. Animals that show signs of chronic pain or distress will be treated with analgesics. Any unexpected exhibition of signs of pain or discomfort will be treated in consultation with the LARC veterinary staff.
Acute experiments are conducted under general anesthesia and the animals are monitored throughout the experiment for signs of pain that may require adjustment of the anesthetic dose. These animals do not recover from anesthesia.

PARTIAL DEAFENING EXPERIMENTS:
Animals are partially and unilaterally deafened in the 1 to 4 kHz range to create a mild to moderate degree of impairment (as described in section G). In our previous work this has had no impact on the animal's behavior as the loss is mild, confined to one ear, and limited in the total frequency range of hearing in the animal.

POSITIVE PROCEDURAL TECHNIQUES
Primates are conveyed to the behavioral laboratory in specially designed home-cage nest boxes or primate chairs, to minimize stress in transport. All squirrel monkey behavioral studies are conducted with positive reinforcing rewards (food, juice), using minimum required deprivation schedules to insure that the deprivation does not put these animals at risk or unacceptable discomfort. Animals are rewarded participants in all applied behavioral procedures. For all chronic electrophysiological studies, we have designed chronic appliances with the objective of minimizing their impacts on each animal's daily life. In behavioral procedures that require fixed head positioning, such procedures are designed so that head and body postures are comfortable positions that occur in the animal's natural environment. Further, fixed head positions are associated with positive reinforcements (e.g., water or juice rewards) in behavioral training sessions. An experimental session is terminated if the monkey exhibits signs of distress (e.g., atypical vocalizations) or if it becomes unusually agitated or struggles in the apparatus (the animals are monitored via closed-circuit TV. This monitoring is live and no video recordings are made).

You do not have USDA Pain Category C Animals.

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You have USDA Pain Category D Animals that would receive relief from pain, discomfort or distress.

You do not have USDA Pain Category E Animals.

F.1. IACUC/LARC Standard Procedures (see Appendix - Section F for full text)

Monkey, Squirrel
Standard Procedures

The techniques listed below have established IACUC/LARC Standard Procedures defined for them. You must follow the procedures exactly as written or you will be out of compliance. Any variation must be described and justified in this text box and approved by the IACUC.

F.2. UCSF Core Facilities

G. Procedures Involving Living Animals

Species: Squirrel Monkey Group: D

All the procedures described here are essentially identical to what has been approved in the past, and with which we now have extensive experience.

We perform 2 main classes of experiments:

1) Chronic electrophysiological recordings in awake animals.

2) Acute electrophysiological recordings in anesthetized animals.

Chronic auditory studies explore the responses to stimuli in the awake animal while the animal is listening passively. Acute auditory studies explore the response and cortical representation of specific auditory stimuli across several sensory cortical fields. The purpose is four-fold: a) to evaluate the distributed representation of the stimuli across large segments of auditory cortex; b) to provide exhaustive control data for the stimuli used in the awake paradigm; c) to allow comparison between the anesthetized and awake/wakeful preparation; and d) to make injections of neuroanatomical tracer material to obtain cytoarchitectonic and connectivity information.

The list below covers all procedures to be carried out. There are 2 groups with respect to the procedures in this protocol.

Group 1: Awake, Passive Listening Electrophysiological Studies.

Procedures for this group are:

A1,2) Evaluation of hearing threshold and Partial Hearing Loss;

B) Pre-surgical adaptation for chronic physiological recordings;

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C) Surgery for chronic physiological recordings;

D) Postsurgical adaptation for chronic physiological recordings;

E) Surgery for acute physiological recordings (*optional)*;

F) Euthanasia.

Group 2: Acute Electrophysiological Studies.

Procedures for this group are:

A1,2) Evaluation of hearing threshold and Partial Hearing Loss;

E) Surgery for acute physiological recordings;

F) Surgery for acute slice preparation;

G) Euthanasia.

Animals that participate in chronic recordings may also be used as subjects in acute experiments when the chronic recordings are complete. Components of minor or major surgical procedures may be combined in order to reduce the total number of procedures for a particular animal or to reduce the total time it will take to perform the procedures (subject to the time constraints of Section H).

PROCEDURAL DETAILS:

A1. EVALUATION OF HEARING THRESHOLD

Otoscopy (the visual examination of the ear canal) may be performed under injectable ketamine:midazolam or inhalation isoflurane anesthesia (after ketamine sedation)

to exclude the possibility of a middle ear infection or fluid accumulation. For dosages of pharmacological agents, recovery, monitoring and documentation see Section I. Three superficial scalp electrodes may be attached with adhesive, or placed with the use of small hypodermic needles to record Acoustic Brainstem Responses (ABRs). Evoked responses to auditory stimuli are recorded to determine hearing thresholds (still under anesthesia). The stimuli are within decibel ranges that do not induce hearing loss. This procedure takes ~45-60 minutes. Afterwards, the recording electrodes are removed; the monkey is recovered and returned to its home cage. No post-procedural analgesia is necessary.

A2 INDUCTION OF PARTIAL HEARING LOSS

Some animals will undergo a procedure to induce partial hearing loss in the right ear of the animal. Asymmetric sensory-neural hearing loss (SNHL) is induced in young adult squirrel monkeys (Saimiri sciureus) by exposing the right ear of the animal to loud sounds [redacted] General anesthesia is maintained with isoflurane and/or ketamine/xylazine, ketamine/midazolam. The cardiovascular system is supported with lactated ringers delivered at 6-8/ml/kg/h, and core temperature is maintained at ~38C.

The left ear is protected by placing an occlusive foam earplug (E-A-R Classic, Aearo Corporation, Indianapolis, IN) into the external auditory canal. Sound attenuation conferred by the E-A-R insert is rated to be 23 dB at 1 kHz by the manufacturer and National Acoustics Laboratory (Sydney, NSW, Australia). [Protection from

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exposure to higher frequencies is even better with an increase of ~3dB per octave above 1 kHz]. The left external meatus is occluded further by packing the concha cavum with mineral oil soaked cotton pledgets, which provides an additional incremental sound attenuation between ~6-16dB. Sensorineural hearing loss induction directed at the right ear is performed in a sound-attenuating chamber (IAC, Bronx, NY) fitted with a video surveillance system. Eardrums will be checked otoscopically before and after the procedures. A sine wave oscillator (General Radio 1396-A, General Radio Company, West Concord, MA) generates the continuous 1, 2, or 4 kHz signal used for acoustic overstimulation at 136 dB SPL for 3 hours. The protective rating at 1 kHz is the minimum value. After the exposure the animal is given buprenorphine and/or meloxicam. This regimen has been shown before to induce the desired mild to moderate level of SNHL [redacted], A power amplifier (MA2400, Crown Audio, Elkhart, IN) conditions the 1 input signal for sound delivery through a high performance midrange driver (M4, Community Professional Loudspeakers, Chester, PA) that is positioned 5-6cm from the tragus of the exposed ear, along the interaural line. The intensity level of the tone is measured at the right lateral ear canal with a sound meter (Brüel and Kjær 2209, Brüel and Kjær, Norcross, GA).

Several weeks after hearing loss induction, tone bursts (linear rise/fall 1 msec, total duration 17 msec, interstimulus interval 35 msec; 0.5, 1, 2, 3, 4, 6 and 8 kHz) will be used to determine the degree of SNHL by assessing the peripheral auditory brainstem response (ABR) thresholds under anesthesia as described in Section A1. This procedure will take ~1 hour.

The procedure itself is performed under anesthesia to avoid potential stress induced by the loud sounds. The after-effect, a mild to moderate partial hearing loss in one ear only, is not stressful for the animals since it does not deprive them from perceiving and reacting to their normal sound environment.

Experiments involving these animals are largely matched to experiments done in normal hearing animals to investigate the misalignment of interaural cortical response maps in asymmetric hearing loss and to document the plastic changes in central processing of sound stimuli arriving from the nominally better hearing ear that may account for progressive realignment of both interaural frequency and threshold maps.

Ref: [redacted]

B. PRE-SURGICAL ADAPTATION FOR CHRONIC PHYSIOLOGICAL RECORDINGS:

Housing: In order to provide a richer social environment for the monkeys, they will be socially housed whenever possible and participate in an environmental enrichment program. Details of the housing and enrichment programs are coordinated between investigators and LARC staff to avoid potential negative interactions between the experimental training procedures and the enrichment program.

Transport: Each monkey is trained to enter a transport box.

Chairing: Adaptation to sitting in the primate chair goes as follows: We begin by bringing a monkey to the lab and allowing it to explore the restraint chair and to start the shaping process for sitting in the primate chair. Exploration of the chair is reinforced by offering treats such as mealworms when the animal is inside the chair. The monkey is then trained to position itself in a neck yoke by one or a combination of the following methods, all providing positive rewards (treats):

1.The monkey is offered preferred rewards when it spontaneously and correctly positions its head.

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2. The monkey is encouraged to position its head in the yoke by showing or placing a leather glove inside or adjacent to the chair. The monkey is immediately offered preferred treats upon correct positioning.

3. The investigator manually positions the monkey in the yoke and immediately offers preferred treats upon correct positioning. The duration of yoking is gradually increased over the period of one to two weeks.

Our goal is to quickly minimize handling of the animal and thereby reduce stress while achieving this necessary step in our training.

Reinforcement: In general, behavioral shaping involves reinforcing successive, increasingly accurate approximations of a response desired by the trainer. A "reinforcer" is any event that strengthens or increases the behavior it follows. There are two kinds of reinforcers: 1. Positive reinforcers are favorable events or outcomes that are presented after the behavior (e.g., food treats). 2. Negative reinforcers involve the removal of (mildly) unfavorable events or outcomes after the display of the desired behavior (e.g., presentation and removal of an animal-handling glove). In both of these cases of reinforcement, the desired behavior increases. In our case, during the shaping phase, the animal is rewarded with a treat when positioning its head/body correctly in the chair, even if a negative reinforcer was used. Therefore, both reinforcement-schemes combined provide positive reinforcement and the most efficacious way of shaping the behavior. At the conclusion of yoke training, a monkey should enter the chair spontaneously, place its head in the upper chair section, and sit in the chair while tolerating the presence of the yoke. We have achieved this in a large number of New World monkeys over that last decade, including several Squirrel monkeys.

C. SURGERY FOR CHRONIC PHYSIOLOGICAL RECORDINGS

Number: Surgical procedures may be combined in order to reduce the total number of procedures for a particular animal or to reduce the total time it will take to perform the procedures.

Duration: The majority of our surgical procedures last less than 3 hours, but can take up to approximately 6 hours. Alternatively, surgical procedures may be split into separate sessions when there is the possibility that combined procedures will keep the animals anesthetized too long, or to avoid prolonged maintenance of a recording chamber implant that will not be used for several months.

Fasting: Monkeys are fasted from solid food from 1 to16 hours before surgery. TANG or other fluids containing nutrients may be made available to the monkeys during this solid-food fasting period to avoid hypoglycemia. The animals are diurnal and usually do not feed during the night. Therefore, starting the fasting at the beginning of the dark cycle the evening before a major surgery does not constitute unusual stress. For details on monitoring and documentation, see Section J.

Induction: The animals will be first sedated with Ketamine or Ketamine/Midazolam. Glycopyrrolate will be given to reduce secretions and famotidine to reduce emesis. We will then induce anesthesia with inhaled Isoflurane or Sevoflurane through a mask and intubate the monkey. All animals on inhaled anesthetics will be intubated and they may be ventilated. The animal will be maintained at a surgical plane of anesthesia. For dosages of pharmacological agents, see Section I. Once areflexic, the monkey is placed in a stereotaxic apparatus or the head is stabilized with a specially designed head holder.

Some animals may receive an alternate induction protocol per LARC veterinarians to reduce handling stress. Alternate protocol includes SR buprenorphine, induction via inhaled isoflurane (box induction). "To effect" amount of IV ketamine/midazolam to allow intubation. Glycopyrrolate as indicated. Famotidine or maropitant may also be used prophylactically to reduce GI irritation. Maintenance anesthesia is via isoflurane as described above

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Fluid Support: An IV line is placed into the saphenous vein or another vein and fluid support is delivered. For shorter procedures or in the unlikely case that no IV line can be placed, fluid will be provided SQ.

Maintenance: Anesthesia will be maintained with Isoflurane or Sevoflurane. We will monitor the depth of anesthesia by measuring the monkeys EKG, rectal temperature, observing rate of respirations, and by ensuring that the animal does not respond to surgical stimuli. Blood pressure, 0_2 saturation and $C0_2$ will be monitored if possible. Thermal support will be provided and temperature monitored.

Implantation of head post for stabilization (Major):

Purpose: The purpose of this implant is to stabilize the monkey's head during electrophysiological experiments in a way that is comfortable for the animal and safe for the investigators. It is essential to secure the monkey's head during the experiments, because this is the only way to record from neurons with manipulable microelectrodes that are inserted into the auditory cortex (AC) in the awake monkey. Implantable (ie, non-manipulable) electrodes severely restrict the number of recording sites that can be sampled and are therefore unsuitable for the planned studies.

Procedure: The appliance to fix the head position consists of an approximately 2 by 3 cm^2 base plate with one vertical post. The base plate is designed to conform to the curvature of the monkey's head. An incision is made in the scalp and muscle and periosteum are cleared to expose the skull. The area is cleaned with sterile saline and/or hydrogen peroxide. With a hand drill, holes are drilled into the skull to allow bone screws to seat the bone. The head restraint appliance is secured to the skull with from 4 to12 titanium bone screws that do not penetrate the dura. A bone cement/acrylic is used to cement the screws to the base plate and to secure the base plate to the skull. Osteogenic integration of titanium screws will take four or more weeks. The surgical wound area is sutured around the base plate. Cylinder and head post implants will be checked and cleaned at least once a week with additional cleanings as needed. When animals are brought up for cleaning of the cylinder implant (1-3 times a week) the post and post margins are inspected and cleaned as needed.

Implantation of cylindrical recording chamber on bone overlying auditory cortex for electrophysiological access (Major):

Purpose: The purpose of the recording chamber implant (also referred to as a "cylinder" or "ring" below) is to allow chronic access to the bone overlaying the auditory cortex (AC) during electrophysiological recording. We plan to record from the site of the first ring for up to several months. The implantation of a second stainless steel/titanium ring on the bone overlying the contralateral AC may be performed at a later time (see below). We may implant the contralateral ring while the initial ring is still functional, but will not perform a craniotomy in the second ring until we have ceased recording from the initial ring.

Procedure: The recording cylinder implantation will occur four weeks or more after head post implantation. The surgical site is located slightly anterior and dorsal to the external ear. Following a vertical incision at the site, we clear muscle and periosteum from the bone. The area of contact is cleaned with sterile saline and/or hydrogen peroxide. A stainless steel/titanium ring (approximate dimensions: diameter 8-15 mm, height from about 5 to 8 mm, wall thickness: ~1 mm) is attached to the lateral skull overlaying the AC with 2 to 5 titanium bone screws and a small amount of bone cement/acrylic. Dental cement may be used on skull surfaces to prevent fluid seeping from inside or outside the ring and to attach to and protect the structural integrity of the bone. If necessary, small bone screws may be inserted into the exposed bone within the ring to anchor the dental cement. As little skin as possible will be removed to guarantee a smooth approximation of the skin to the two appliances (head post and ring). The surgical wound area is sutured to approximate the implant.

For hygienic purposes, the ring is closed with a cap or filled with sterile silicone elastomer or sterile petrolatum ointment to retard the growth of granulation tissue [redacted]

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[redacted]. After the cylinder is placed, we wait at least 3 days before placing burr holes or performing electrophysiology if a burr hole was performed during ring implant surgery. Implants will be checked and, if indicated, cleaned once a week. Note that as a precondition for all physiological experimentation, the cylinder implant is inspected and cleaned prior to electrode insertion.

Endpoint: When recording sites within the first ring implant are exhausted, a second ring may be implanted over the contralateral cortex. In order to avoid maintenance of the unused first ring and possible risks of infection over the long periods of time required for behavioral adaptation and electrophysiological recordings, we may remove the first ring, close the wound, and attach the second ring in a single surgical procedure to minimize the number of surgeries, or as a separate surgery to minimize surgical time.

Burr Hole Placement (Major):

Purpose: The purpose of this procedure is to provide access to the auditory cortex through a small craniotomy within the ring implant. This allows microelectrode recordings of neurons from the auditory cortex. Over a period of several weeks to several months, recordings may be obtained from several sites within the ring, thereby producing a functional map of the auditory cortex. A burr hole procedure may be performed immediately following the positioning of the ring and/or weeks later to position holes over different regions of auditory cortex.

Procedure: Under anesthesia, a hand drill, pin vise, dental cutting burr and/or trephine is used to expose the dura by drilling 1-4 holes in the skull inside the ring. The diameter of each drill hole is 1-3 mm. If necessary in order to access the auditory cortex, any hole may be enlarged at this or a later time (for example when a burr hole is located above a sulcus). After exploiting useable sites for recordings within a single burr hole, another hole may be uncovered, an existing hole expanded (see below), or it may be permanently sealed with bone cement or may be left to close by osteogenesis. Prior to drilling new burr holes, regions of the bone within the ring may be slightly thinned with a drill to flatten the surface and/or improve visibility of underlying structures (vessels, sulci). Bone stability is not compromised since the ring is secured from the outside to stable, normal bone. The timing between different burr hole surgeries will be at least 3 days (if the previous burr hole is unsuitable) but is usually several weeks.

Deferred dural opening and recording: No dural incision is made during the surgery, the craniotomy over the dura is plugged with an antibiotic ointment, the ring is capped, and the monkey recovers from anesthesia in the surgical suite and is then returned to its home cage. The monkey is restrained at a later date in the primate chair, and using aseptic techniques, the area enclosed within the ring is cleaned with dilute Betadine/Novalsan, and the burr hole craniotomy is cleaned to expose the dura and a drop of 1% lidocaine is applied to the dura. A small incision is then made in the dura. Historically, this procedure is well-tolerated in the awake animal, judging from the absence of any signs of discomfort. A microdrive-mounted microelectrode is advanced through the cut in the dura into the auditory cortex. The purpose of this recording episode is to characterize neurons encountered by a penetration across laminae within AI. This procedure will last about 3-5 hours. Afterwards the ring implant will be cleaned and capped, and the monkey will be returned to its home cage.

Expansion: If the existing burr holes do not provide viable recordings within the expanse of auditory cortex accessible within the cylinder implant, a burr hole may be widened with sterile instruments. This minor, aseptic procedure performed within the recording chamber will be conducted after applying 1% lidocaine to the existing craniotomy and an oral analgesic will be administered after returning to the cage. In our previous experience during the past 3 years this minor procedure has been well-tolerated with no noticeable reactions by the awake animals.

Only one dural incision is usually required for each burr hole, i.e., multiple electrode penetrations are typically made within a single dural incision. Because the dural incision may close up, however, multiple incisions, or

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enlargements of the original incision may be made after application of a drop of lidocaine. Topical lidocaine will also be used when electrodes are advanced through uncut dura.

Burr holes initially are from 1 to 3 mm, and are of necessity limited by the size of the cylinder implant itself (approximately 1 cm in diameter). It should be noted that expanded burr holes need not be circular in shape, so we can limit the expansion along a particular axis to minimize the overall exposure while providing sufficient access to the cortex. Thus, for example, a hole could be up to 8 mm long, but only 2 -3 mm wide. For reference, previous research suggests that a burr hole diameter of 8 mm would span the entirety of auditory cortex in this species.

Recording Procedures: All recordings begin with a thorough cleaning of the cylinder implant. A microelectrode is advanced into the cortex to permit neuronal recording during each experimental session. The recording electrode may be stabilized by applying agarose in sterile saline to the burr hole. Agarose is removed at the end of recordings. Microelectrodes coated with a lipophilic fluorescent dye (DiI, DiO, DiI-C5 or Fast Blue; Invitrogen Corp.) may be used to mark and identify the tracks and region of brain tissue where recordings have been made. The dyes do not produce either short-term or long-term adverse effects [redacted].

Recording Chamber Maintenance: With the head restrained, the area inside the ring is cleaned with diluted Betadine or Novalsan and any granulation tissue in the burr hole is removed with fine, sterile surgical instruments. Lidocaine is applied prior to removal of granulation tissue After conclusion of the daily recording session (duration up to ~5 hours, including 15 to 120 minutes required to isolate and characterize neurons), the microelectrode is withdrawn from the cortex, the opening over the dura is plugged with an antibiotic ointment, and the ring and surrounding area is cleaned and secured as described above.

Endpoints: After repeated recordings in one burr hole have been concluded, one of the following happens: *1*) The hole is allowed to close by osteogenesis, or permanently closed with dental cement. In this case, another burr hole is opened and the dura resected (see above). Recordings then commence at the new site. *2*) The hole is expanded as described above. Recordings then commence in the newly exposed region of cortex. *3*) The current burr holes will all be permanently closed and new holes will be opened in a repeat of the major burr hole placement procedure (see above under C).

Implant repair or reposition (Major/Minor):

It may be necessary to repair, replace, stabilize, reposition or remove implant(s).

Repair: Minor repairs may involve applying or removing bone cement to exposed bone or to an implant (no anesthesia), or removing invasive tissue on the border of an implant (under local anesthesia for minor tissue removal or general anesthesia for more extensive tissue removal). The medical feasibility to repair, replace, stabilize, or reposition a device will be discussed with veterinary staff. Repairs will be implemented as required by the nature of the implant defect. For example, the screws used to hold the cylinder in place can loosen from the bone, in which case new screw holes may be required to stabilize the implant. After repair surgery, we wait at least 3 days before resuming physiology.

Recording Chamber Repositioning: Repositioning of the recording chamber may require a shift of the implant location by less than the diameter of the implant. This is indicated if the initial positioning of the implant does not allow access to the target region in auditory cortex (this will not be evident until after placing several recording penetrations into the cortex through any of the burr holes; there are no unequivocal landmarks on the skull or stereotactic coordinates to indicate the precise location of the auditory cortex nor does MRI help in locating an area for recording since location variability between individual animals is high). The implant repositioning will typically not require major incisions or removal of large amounts of skin or muscle tissue since the original surgery will have already made the area of interest largely accessible. These procedures will follow

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the same surgical steps as outlined above for the initial placement and/or removal of the devices. No more than two adjustments to the cylinder position in each animal will be conducted. Time between repositioning and returning the animal to study is at least 3 days (similar to the original ring implantation).

D. POST-SURGICAL ADAPTATION FOR CHRONIC PHYSIOLOGICAL RECORDINGS

The animal resumes the acclimatization training described in section B after a post-surgical rest of at least one week following a major surgical procedure. Adaptation to head restraint may begin after a minimum period of 4 weeks for recovery from headpost implant surgery. The animal is placed in the primate chair, and after the head is restrained by connecting the head-mounted appliance to a rod that is attached to the frame of the chair, the monkey's attention to restraint is distracted by offering preferred treats. Because the monkey has extensive prior experience being handled, trained and rewarded, adaptation to head restraint is typically not difficult for the monkey. Nevertheless, a conservative approach is used for this important stage of training. Preferred treats and additional juice rewards are offered to encourage the animal, and the duration of head restraint is increased gradually over several sessions to avoid distress to the monkey.

On recording or training days (or at least once weekly), the implants and wound sites will be inspected, and if needed, cleaned with normal saline and/or diluted Betadine and/or Novalsan. If required, topical antibiotics and/or vitamin A&D ointment may be applied to implant margins. During inspection and cleaning of the area enclosed by the ring implant, granulation tissue may be removed mechanically and a small amount of suspension steroid (Triamcinolone or similar), sterile silicone elastomer, sterile petrolatum ointment, or vitamin A&D ointment (emollient) may be topically applied to retard its growth.

E. SURGERY FOR ACUTE RECORDINGS

Mapping and Brain Tracer Injection Experiments

Some animals (potentially including those at the end of the recording sessions described in section C) will undergo an acute, terminal recording session followed by euthanasia. The goal of acute electrophysiological experiments is to derive information about receptive field properties of auditory forebrain neurons to reconstruct response maps of several cortical fields. Brain tracer injections permit the study of the relationship between brain micro-circuitry and functional response properties.

Duration: 2 to 5 days

Fasting: Monkeys are fasted from solid food from 1 to16 hours before surgery. TANG or other fluids containing nutrients may be available to the monkeys during this solid-food fasting period to avoid hypoglycemia. The animals are diurnal and usually do not feed during the night. Therefore, starting the fasting at the beginning of the dark cycle the evening before a major surgery does not constitute unusual stress. For details on monitoring and documentation, see Section J.

Induction: Dexamethasone may be given approximately 24h prior to SX, and prophylactically to manage brain edema during the procedure. The animals will be first sedated with Ketamine, Ketamine/Midazolam or Ketamine/Diazepam. Glycopyrrolate will be given to reduce secretions and famotidine to reduce emesis. We will then induce anesthesia with Pentobarbital. Animals will intubated and may be ventilated. The animal will be maintained at a surgical plane of anesthesia. For dosages of pharmacological agents, see Section I. Once areflexic, the monkey is placed in a stereotaxic apparatus or the head is stabilized with a specially designed head holder. The airway is kept moist with a stream of moist air to reduce the formation of mucous plugs (a narrow plastic tube is positioned near the end of the endotracheal tube and humidified medical air is delivered to the animal). The anesthetic agent Pentobarbital is used during the surgical procedure because acute use of high concentrations of isoflurane, necessary for surgical manipulations, causes long-lasting and severe depression of cortical activity

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[redacted] precluding unbiased electrophysiological exploration of the auditory cortex. After conclusion of the surgery, pentobarbitol is replaced by a mixture of ketamine and diazepam (to effect), a regimen that preserves more normal spontaneous and driven activity of cortical neurons.

[redacted]

Tracheotomy: A vertical skin incision is made over the trachea. The strap muscles are retracted laterally, and the tracheal lumen is entered. A modified endotracheal tube is secured to the trachea with suture and then the incision is closed with suture and/or staples. Tracheotomy improves pulmonary mechanics by reducing dead space and permits direct access to the tracheobronchial tree to remove mucous plugs. Alternatively, endotracheal intubation may be used.

Fluid Support: An IV line is placed into the saphenous vein or another vein and fluid support is delivered. In the unlikely case that no IV line can be placed, fluid will be provided SQ.

Maintenance: Anesthesia will be maintained with Pentobarbital or Ketamine/Diazepam. The depth of anesthesia is continuously monitored and the monkey is kept areflexic for the full duration of acute experiments (2 to 5 days). We will monitor breathing rate, heart rate, and neural responsiveness. A water-circulating warming blanket with feedback control and/or heating pads (such as "safe-and-warms") will be used to maintain proper body temperature. An antibiotic is administered every 3 hours to prevent significant infection. Mechanical ventilation may be used to control respiration and $C0_2$.

Craniotomy and Electrophysiological Brain Mapping and Brain Tracer Injections (duration: 2-5 days):

The animal is placed in a stereotaxic or orbital/palatal head holder. Evoked responses to auditory stimuli quantify any potential level of hearing loss. The scalp is injected with Bupivacaine to augment general anesthesia. An incision over the temporoparietal scalp is made and the underlying soft tissue and muscle are retracted to expose the lateral skull. Burr holes are placed and a bone plate is removed over the auditory forebrain. The dura is entered and reflected to reveal the auditory cortex. Recording electrodes are introduced to develop a response profile to an auditory stimulus battery. We use two types of electrodes during acute recordings: (1) penetrating microelectrodes; and (2) surface electrocorticographic (ECoG) arrays. Microelectrodes allow for acquisition of neural data across the depth of auditory cortex within a cortical field, while ECoG arrays allow for acquisition of neural data across the cortical surface from multiple cortical fields simultaneously and without perforation of the cortical tissue. For combined physiological and anatomical studies, a limited response map that marks the functional borders but minimizes cellular distortion is derived in preparation for brain tracer injections. A combination of anterograde (biotinylated dextran amines) and retrograde (wheat germ agglutinin (WGA) apo horseradish (HRP), gold labeled WGA-apo-HRP, cholera toxin ß subunit tracers are then introduced into brain regions of interest. In the second hemisphere, a complete response map may be derived for an auditory stimulus battery.

At the end of these acute experiments, the brain may be marked with intra-cortical electrolytic lesions or fluorescent dye, to permit the spatial registration of mapping information with cyto-architecture and laminar depth. After recordings are completed, the animal is euthanized and then perfused by administering a lethal dose of Pentobarbital (150 mg/kg) followed by a thoracotomy. This is then followed by intra-cardiac sequential buffered saline and glutaraldehyde/formaldehyde systemic perfusion.

F. SURGERY FOR ACUTE BRAIN SLICE EXPERIMENTS

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At the end of some acute recording sessions, animals will be more deeply anesthetized by an additional dose of Pentobarbital or Ketamine/Diazepam and brain tissue will be excised for slice physiology.

Duration: 2 to 4 hours

In preparation for this procedure, the animal will initially undergo bilateral acute brain electrophysiology as described in section "E" above to determine the location of the auditory cortex. In this procedure, no tracers will be injected. Once this has been done the animal will be more deeply anesthetized and the craniotomies will be further enlarged to gain access to the auditory cortex and surrounding areas so that sufficient tissue can be excised for complete auditory cortex removal along with associated surrounding and deeper layers of the brain. The tissue will be excised in two sessions separated by about an hour. For this reason the animal cannot be euthanized and then the tissue removed because the hour long wait would not result in viable tissue from the second excision. Once the tissue has been excised the animal will be euthanized by an overdose of Pentobarbital (Fatal Plus) followed by a bilateral thoracotomy.

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G. EUTHANASIA

Unforeseen complications (e.g., infection) resulting in sustained lethargy, loss of appetite or poor grooming that are unresponsive to intervention by the veterinary staff may indicate that the animal should be euthanized. Euthanasia of chronic animals will only be performed after veterinary consultation. For euthanasia of acute and chronic animals, an overdose of Pentobarbital is administered followed by a bilateral thoracotomy and, optionally systemic perfusion by intracardiac sequential buffered saline and glutaraldehyde/formaldehyde.

Note on anesthetics/analgesics:

H. Surgery and Post-Operative Care

Squirrel Monkey	
H.1 Surgery	
Surgery Performed:	Yes
Surgery Type:	Survival (Single), Survival (Multiple), Non-Survival
List each surgical procedure and the anticipated duration of the surgery:	Major survival surgeries (head restraint, recording ring implantation) last 3-6 hours. Preparatory surgery for acute recordings typically last 3 - 6 hours but the acute physiological recording procedures last 2 - 5 days.
If there will be multiple survival surgeries on the same animal, justify the need.	1) In section G (sub-section C), a procedure is described to allow recording of cortical neurons by placing small burr holes in the skull in an anesthetized animal. This procedure was selected because it exposes the least amount of cortex required for recording, minimizes cortical edema and reduces the likelihood of infection that may occur with large, chronic exposures of cortex in animal models. To achieve our scientific objectives, small exposures may be made

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	sequentially over a period of weeks to months at locations corresponding to different regions of auditory cortex. The initial placing of burr holes in the first or second ring may not allow access to all desired portion of auditory cortex. Placement of additional burr holes will be undertaken under anesthesia and sterile conditions. To minimize the number of these additional surgeries, each procedure will place 1-4 burr holes. The number of additional burr hole procedures is limited by the size of the exposed skull area and is not expected to exceed three per hemisphere. For the animals used in the hearing loss experiments, two cylinders must be implanted concurrently to allow for recording from each hemisphere within the same week, given the longitudinal nature of those experiments. Each cylinder will be implanted during a separate procedure. 2) Implantation of the first ring may be done in the same surgery as implantation of the head restraint. However, if it appears that adaptation to head restraint and behavioral training will be prolonged in an individual monkey, the ring implant may be postponed to avoid prolonged maintenance of the implant margin around the ring. 3) We plan to record from the site of the first ring for up to several months. In order to avoid maintenance of two rings and to reduce possible risks of infection over the long periods of time required for our experiments, we may remove the first ring, surgically close the wound and attach the second ring in a single surgical procedure (2-4 hours). Implanting the second ring will serve the following purposes: 1) maximize the data acquisition per animal, which may also reduce the number of animals used in the study and 2) allow comparison of differences/similarities in mechanisms of signal processing and the effects of learning between the two hemispheres (e.g., right hemisphere dominance in processing of frequency modulated signals). 4) Insertion of electrodes percutaneously in the neck muscles to record muscle tone and activity in order to monitor sleep/wakefulness during recording from the auditory cortex. This measurement allow us to investigate the responses of primary auditory cortex neurons under different states of sleep/wakefulness, and are distinct from the intra-cranial electrode recordings described in Section G. This relatively minor procedure is performed under general anesthesia either at the conclusion of the first or second ring implant described above or at a later time in a separate sterile surgery (1-2 hours) if an animal has been found suitable to participate in this part of the study.

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	5) Additional surgeries may be necessary to repair, replace, reposition, or stabilize a malfunctioning appliance. In our experience over the last 3 years we had two cases of device malfunction that required major surgery for repair, reattachment, or removal.
Will there be multiple major survival surgeries performed on the same animal?	Yes
List each major survival procedure and the duration of time to be allowed between surgeries on the same animal:	1) Head post implantation. After surgery, we wait at least 4 weeks for osteogenic integration to occur before other major surgeries are being performed (ring implant, burr hole placement) 2) Recording cylinder implantation. This will occur 4 weeks or later after head post implantation. After surgery, we wait at least 3 days before placing burr holes or perform electrophysiology if a burr hole was placed during ring implant surgery. 3) Further Burr-hole placements. Contingent on the physiological condition within a burr hole, additional burr holes may have to be placed. The timing between different burr hole surgeries will be at least 3 days (if the previous burr hole is unsuitable) but usually several weeks to months. 4) Implant repair. After repair surgery we wait at least 3 days before resuming physiology. 5) Recording Chamber repositioning. No more than two of these for any animal will be conducted. Time between repositioning and returning the animal to study is at least 3 days (similar to the original ring implantation).
H.2 Post-Operative Care:	
How long will the animals survive after surgery?	The animals will survive as long as they remain in good health and as long as we can effectively collect reliable data from them. Any post operative concerns with the implants are brought to the attention of veterinary staff and their recommendations and treatment methodologies (e.g antibiotics) are implemented.
Describe post-operative care and the frequency of monitoring in the days or weeks until the animals recover from the surgery (e.g. wound care, monitoring for infection and post-op pain)	It usually takes 10-15 minutes after a major surgical procedure under isoflurane anesthesia and ~20-40 minutes after minor surgical procedures under ketamine:benzodiazepene combinations before the animal is righting, and movements start. Full recovery will usually take ~1.5-2 hours. Monkeys are kept warm during recovery (heat lamp, blanket, or heating pad). Monitoring probes will be removed as soon as they may interfere with the animal's recovery or safety. The IV line is kept clear, and fluid delivery is continued, as long as possible. Once the animal is awake and able to support it's weight it is placed in the monkey chair and brought to the lab where it remains until it is fully ambulatory and can be returned to it's home cage.

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	Alternative recovery from surgery will take place in the surgical suite (OR) until the monkey is fully able to support its weight and it is ambulatory. Post-surgical monitoring is verified by entries in the medical record, and includes at least one more monitoring before the end of the day of surgery, and at least twice a day thereafter until resolved. Because the animals are awake, vital signs are not monitored per se. Instead, the monkey's behavior, eating, drinking, and urinary and fecal output are monitored by observation. Any complications after recovery surgeries will be discussed with the veterinary staff. Meloxicam is administered perioperatively of major survival surgery, on the first morning following the surgery, and on the second morning following surgery. Thereafter administration will be approximately q.24 hours if there is evidence of pain. Buprenorphine will be given (SQ or IM) peri-operatively in major survival surgeries and a second is given in the afternoon on the day of the surgery and subsequent doses may be given twice daily as needed for post-operative pain. Signs of pain include guarding, lethargy, restlessness, abnormal vocalizations or movements, food refusal. Lidocaine is applied at the site of skin incisions and for opening and/or needle penetration of the dura and for burr hole expansions. Meloxicam is administered following burr hole expansion. For cleaning and maintenance of the implants and wound sites, please refer to section G (sub-section D).
Will you follow IACUC/LARC Guidelines for Post-Operative Analgesia?	Yes
Will you follow IACUC/LARC Guidelines for Animal Surgery:	Yes

I. Pre-Anesthetics and Anesthetics, Neuromuscular Blocking Drugs, Therapeutics, Analgesics and Experimental Agents

I.1

Monkey, Squirrel: Pre-Anesthetics and Anesthetics

Agent	Dose Range (mg/kg)	Route	Frequency / Total Duration	Recover From Agent	Use IACUC Monitor Form	Anesthesia Recovery Time	Pharmaceutical grade
Isoflurane	0.5-5%	Inhalation	Continuously during procedure; induction and option for surgical portion of	Yes	Yes	1.5 - 2 hours	Yes

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			acute procedures. For acute procedure, animals will not recover.				
Ketamine	10-25 mg/kg	IM, IV	Single dose, PRN for sedation	Yes	Yes	About 15 minutes	Yes
Ketamine + Diazepam	20 mg/kg/hr + 0.006 - 0.02mg/kg/hr	IV	about every 15 min - see attached reference	No	Yes	animal will not recover. reference for anesthesia regimen attached to documents section.	Yes
Ketamine + Midazolam	10-25 mg/kg + 0.05-0.2mg/kg	IM, IV	for induction, single dose, PRN	Yes	Yes	about 15 minutes	Yes
Ketamine + Xylazine	10 - 20 mg/kg & 0.25 – 3.0	IM or SC	As needed	Yes	No	30 to 60 minutes	Yes
Pentobarbital	150 - 200 mg/kg	IP	once for euthanasia or terminal perfusion	No	No	animal will not recover	Yes
Pentobarbital	5-30 mg/kg, to effect	IV	Divided doses titrated to effect for acute recording procedures. May also be used for surgical portion of acute procedures, instead of Isoflurane.	No	No		Yes
Sevoflurane	1-8%	Inhalation	Continuously during procedure	Yes	Yes	same as isoflurane	Yes

Monkey, Squirrel: Pre-Anesthetics and Anesthetics Monitoring Details					
Agent	**Variable Monitored**	**Monitoring Frequency [Anesthesia]**	**Monitoring Frequency [Recovery]**	**Doc/Charting Frequency [Anesthesia]**	**Doc/Charting Frequency [Recovery]**

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Isoflurane	Heart rate (EKG)	Cont.	30 min until righting reflexes returned	15-30min. We will use the keck Surgery forms.	60-90min until righting reflexes returned
	O2- or CO2-saturation (in procedures with intubation)	Cont.		15-30 min	
	reflexes (paw pinch until anesthetized, or responsiveness to surgical manipulation)	15-30 min	30 min until righting reflexes returned	15-30 min	60-90min until righting reflexes returned
	Respiration rate	Cont.	30 min until righting reflexes returned	15-30 min	60-90min until righting reflexes returned
	Temperature	Cont.	30 min until righting reflexes returned	15-30 min	60-90min until righting reflexes returned
Ketamine	level of arousal	every 30 minutes	every 30-60 min until righting reflexes returned	every 15-30 min	every 30-60min until righting reflexes returned
	Respiration rate	Every 30 minutes	30-60 min until righting reflexes returned	every 15-30 minutes	Every 30-60min until righting reflexes returned
Ketamine + Diazepam	same as ketamine	same as ketamine	no recovery	same as ketamine	no recovery
Ketamine + Midazolam	same as Ketamine	same as Ketamine	same as Ketamine	same as Ketamine	same as Ketamine
Ketamine + Xylazine	same as ketamine	same as ketamine	same as ketamine	same as ketamine	same as ketamine
Pentobarbital	Heart rate (EKG)	continuously		Every 30-60 minutes, we will use the IACUC Anesthesia Monitoring Form; during the recording procedures we will use forms custom-made for our needs.	
	Reflexes (paw pinch or cornea reflex)	every 15-30 min during surgery; every 30-60 minutes during recording period		every 30-60 min after surgery is complete (during recording session)	
	Respiration rate	Continuously or every 15-30 min during surgery; every 30-60 min		Every 30-60 min.	

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		after surgery is complete (during recording session)			
	Temperature	Every 30 min during surgery; every 30-60 min after surgery is complete (during recording session)		every 30-60 min.	

I.3				
Monkey, Squirrel: Therapeutic				
Agent	Dose Range (mg/kg)	Route	Frequency / Total Duration	Pharmaceutical grade
Atropine	0.04 mg/kg	IV,SQ	For bradycardia; single dose, if required	Yes
Bacitracin Zn & Polymyxin B Sulfate Ophthalmic Ointment	ribbon	topical	as needed for ring implant (recording chamber) management	Yes
Betamethasone	a few qtts	topical	in implant and/or wound margins during cleaning; PRN	Yes
Cefazolin	10-20 mg/kg	IV or IM	At start of surgery and every 3 Hrs during acute recordings. Additional antibiotics will be administered as needed in consultation with a veterinarian.	Yes
Chlorhexidine (Nolvasan)	a few qtts	topical	Single dose; PRN for implant margins	Yes
Ciloxin	few qtts, ribbon (ophthalmic)	topical	in implant and/or wound margins during cleaning; PRN	Yes
Ciprofloxacin	1-2 drops (ophthalmic)	topical	as needed for ring implant management	Yes
Dexamethasone	0.25-2 mg/kg 12-24h prior to SX; q12-24h during surgery	IM or SQ; IV	PRN to prevent brain edema during acute experiment	Yes
Dopram	1-3mg/kg	IV	PRN for respiratory depression during anesthesia	Yes
Epinephrine	0.1 to 0.5mg/kg	IV	as needed for cardiac resuscitation	Yes
Famotidine (Pepsid)	0.5mg/kg	IV	at the beginning of survival surgeries (prevent emesis)	Yes
Glycopyrrolate	0.02mg/kg	IV,IM	During induction, PRN	Yes

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Hydrogen Peroxide	(3%); up to 0.5 ml	topical	as needed to clean exposed skull	Yes
Lactated Ringer's Solution	5-8 ml/kg/hr (may add 2.5 Dextrose and/or 20 mEq KCl)	SQ/IV	continuously during surgery and acute recording procedure	Yes
Mannitol	0.25-0.5 g/kg	IV	for prevention and/or reduction of brain edema during acute experiments; PRN	Yes
Maropitant (Cerenia)	1mg/kg	IM	One dose	Yes
Ophthalmic Ointment (artificial tears)	not applicable	topical	As needed for the duration of surgery.	Yes
Ophthalmic Ointment (triple antibiotic)	ribbon	topical	as needed for ring implant (recording chamber) management	Yes
Oxytetracycline	ribbon or drops (ophthalmic)	topical (in ring implant)	as needed	Yes
Polymixin	few qtts, ribbon (ophthalmic)	topical	in implant and/or wound margins during cleaning; PRN	Yes
Triamcinolone	0.05-0.2ml	topical	sufficient to control proliferation of granular tissue associated with implant; PRN	Yes
Trimethoprim-sulfamethoxazole	25 mg/kg	PO	SID one week (post-operative prophylactic antibiotics)	Yes
Vitamin A and D	ribbon (ointment)	topical	in implant and/or wound margins during cleaning	Yes

I.4				
<u>Monkey, Squirrel: Analgesics</u>				
Agent	Dose Range (mg/kg)	Route	Frequency / Total Duration	Pharmaceutical grade
Bupivicaine (Marcaine)	< 6-8 mg/kg	SQ or topical (alternative to Lidocaine)	survival surgery: Single dose prior to incision; non-survival surgery : single-dose prior to incision;	Yes
Buprenorphine (Buprenex)	.01 - .03 mg/kg	SQ, IM	Implant surgeries, new burr hole procedure: first dose shortly before inhalant is turned off; second dose given in the afternoon on the day of the surgery and	Yes

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			subsequent doses are given twice daily as needed for post-operative pain. Signs of pain will be assessed visually in terms of the animal's general appearance, posture, unusual behavior (e.g. guarding, atypical vocalizations), and/or labored breathing. Lab staff will consult with veterinarian if indicated.	
Carprofen (Rimadyl)	2 - 4	SQ, PO	every 24 hours up to 4 days	Yes
Lidocaine (Xylocaine)	from 1 to less than 8 mg/kg total (1% lidocaine)	SQ or topical	prior to dermal or dural incision or burr hole expansion (alternative to Bupivicaine)	Yes
Meloxicam - "metacam"	0.2-0.3 mg/kg	SQ or PO	At the beginning of survival surgery, morning of the first post-surgical day, and morning of the second post-surgical day; Thereafter the animal will receive will meloxicam once daily if signs of pain are present. Signs of pain will be assessed visually in terms of the animal's general appearance, posture, unusual behavior (e.g. guarding, atypical vocalizations), and/or labored breathing. Lab staff will consult with veterinarian if indicated.	Yes
Proparacaine	1-2 drops	topically	may be applied during surgery when eye bars are used	Yes

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I.5						
Monkey, Squirrel: Experimental Agents						
Agent	Dose Range (mg/kg)	Route	Frequency / Total Duration	Hazardous Chemical	Hazardous Material?	Carcinogen?
Wheat-germ apo-horseradish peroxidase	3 µl	intracerebral	Single dose each site		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Gold labeled wheat-germ agglutinin conjugated to horseradish peroxidase	3 µl	intracerebral	Single dose each site		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Biotinylated dextran amines	3 µl	intracerebral	Single dose each site		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
paraformaldehyde and glutaraldehyde in 0.1 M phosphate buffer	Variable concentrations, depending upon histological technique	Intracochlear,transcardial	Once for fixation		Yes	Yes
MSDS URL: http://www.ucmsds.com/?X						
Betadine (Therapeutic Agent)	a few qtts	topical to the site interior to the cylinder.	Single dose; PRN		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Histoacryl	up to 0.5ml	topical	inside ring and at ring margins during cleaning to protect exposed bone or close burr holes		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Lipophylic dyes (DiI, DiO, DiI-C5, Fast Blue)	several micrograms via surface coating of recording electrodes	intracerebral	1-3 penetrations per case		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
cholera toxin beta subunit or cholera toxin beta subunit conjugated to	~3microl	inracerebral	single dose each site		No	No

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gold chlorid particles						
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Agarose	1-5% in saline	topical	to stabilize cortical surface during recordings		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
dental/bone cement	a few ccs	topical/external on bone to secure screws, headpost and recording chamber (ring)	application during major surgeries to attach headpost and recording chamber; until removal of device from animal		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Novalsan	few drops	topical	cleaning of implant		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Kwik-Sil adhesive, silicone elastomer	0.1-1 cc	topical	cover of burr holes in ring implant; applied between recording sessions and chamber cleaning; usually for 1-2 days up to 7 days		No	No
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						
Phosphate buffered paraformaldyde	10% (dosage not calculated as body weight but as percent paraformadehyde in phosphate buffered saline).	Cardiac assisted venous and arterial infusion.	Once as a terminal procedure.		Yes	Yes
MSDS URL: http://130.15.90.245/Chin-Sang%20Lab%20MSDS/Fisher/Paraformaldehyde.pdf						
Auditory stimuli	range expressed in decibels, not to exceed 100dB spl	via loud speaker to the auditory meatus as free field exposure	stimuli range from short duration tones and tonal component stimuli to longer duration ripple stimuli		No	No

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			(at most lasting a few hundred milliseconds. longer stimuli like dynamic moving ripples are typically at moderate pressure levels (60 - 65 dB)			
MSDS URL: http://ehs.ucsf.edu/safety-data-sheet-sds-1						

I.6	
Will you be using non-pharmaceutical grade experimental agents?	Yes
I.7	
Describe why it is necessary to use non-pharmaceutical grade experimental agents:	described tracers and paraformaldehyde are not available as pharmaceutical grade. in the case of paraformaldehyde is it used post-mortem.
I.8	
List the non-pharmaceutical grade agents to be used, justify the need to use these non-pharmaceutical grade agents, and describe pharmaceutical grade alternatives you have considered:	see all experimental agents listed. there are no pharmaceutical grade experimental agents available as they're not used clinically.
I.9	
Describe how you will prepare and store the non-pharmaceutical agents to ensure sterility:	agents in the 'non-pharmaceutical grade' category are prepared using sterile procedures using pharmaceutical grade agents (eg sterile water) where available and filter sterilized where this would not interfere with delivery of the agent.

I.1.1	
All Species: Pre-Anesthetics and Anesthetics	
Will you follow IACUC/LARC Guidelines for Anesthetizing Animals for Research Procedures:	Yes

J. Management and Monitoring of Adverse Effects of Procedures and Experimental Agents

Squirrel Monkey Experimental Group: D

J.1

Adverse Effects: Squirrel Monkey Experimental Group: D

Procedure, Agent or Phenotype	Potential Adverse Effects	Management
Implant sites	Infection, inflammation, bone erosion, or rejection; skin retraction around implant; any bleeding due to excessive growth of granulation tissue; possibility of need for minor surgery to repair	Veterinary consultation; local cleaning and topical or, if necessary, systemic antibiotics.

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Behavioral training	Signs of distress (e.g., unusual agitation or vocalization)	Immediate termination of the distressing stimulus, and a revision of the behavioral paradigm in consultation with veterinary and behavioral staff.
Implant surgery, craniotomy, opening of dura	Postoperative pain	Analgesics (e.g., buprenorphine/meloxicam); veterinary consultation if pain >24 hr post-op
Craniotomy and opening of dura	Postoperative pain	Analgesics (e.g., buprenorphine/meloxicam); veterinary consultation if pain >24 hr post-op
Acute surgery and recording	Infection, respiratory distress and hypotension for acute procedures	Infection: Administration of prophylactic antibiotics at start of surgery and every 3 Hrs during acute recordings. Additional antibiotics will be administered as needed in consultation with a veterinarian. Respiratory distress and hypotension: manually assisted respiration (baging) and/or administration of Dopram, evaluation of anesthetic depth, thermal support and fluid administration; veterinary consultation.
Chronic recordings	Infection following craniotomy and opening of dura.	Systemic antibiotics and/or topical antibiotics after consultation with the veterinarian. Typically after chronic recordings the site is cleaned and treated with systemic antibiotics.
Acute and chronic recording procedures	Hemorrhage and seizures during microelectrode recording	Superifical hemorrhage: chemical or electrial coagulation (small bleeders stop spontaneously within minutes; large superficial hemorrhages have not been encountered in 25 years of experience with cortical recordings); deep hemorrhage: if bleeding does not cease spontaneously, the animal will be euthanized (after consultation with the veterinarians) seizures: have not been encountered in any of our experiments. If it does occur, we will consult with the veterinarian.
All procedures (inclusive)	Seizure	Immediate veterinary consultation
Chronic Study	weight loss	Veterinary consultation if weight drops 15% below initial surgical weight.
Partial hearing loss	None anticipated. The hearing loss is partial and does not prevent the animal from perceiving natural sounds in its environment.	None needed.
Acute/Survival surgeries	Cardiovascular compromise during long-term anesthesia.	Epinephrine

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J.2

Monitoring Parameters: Squirrel Monkey Experimental Group: D

Monitoring Parameters	Frequency	PI/Lab will Document
General signs including abnormal activity/behavior, appetite, excreta, wound site	Implanted animals are monitored each time they are brought to the lab for an experiment or if the animal is not actively involved in experiments they will be monitored at least once a week.	Yes
weight	Implanted animals will be weighed at least once every two weeks or more frequently (at least once a week) if they are not eating or other issues of weight loss are observed. In cases where the animals have been noted to have lost weight, or there is independently motivated concern about their health, we will consult with LARC veterinary staff, and if more regular monitoring of an animal's weight is recommended, we will do so.	Yes
Implant site checks	At least once a week. Documented in the medical record.	Yes

J.3

Humane Endpoints

Respiratory Distress :	No
Lesions unresponsive to treatment :	Yes
Hunched posture :	No
Body Condition Score of 2 or less :	No
15% weight loss if animals are being weighed on a scale :	No
Impaired or decreased mobility :	No
Tumors that ulcerate or interfere with normal function :	Yes
Dehiscence of surgical incision :	No
Neurologic signs which interfere with normal function :	Yes
Reduced grooming and piloerection :	No
Rectal prolapse :	No

J.4

Other Humane Endpoints.

Following the attachment of appliances (head restraint and steel rings) and following the small craniotomies we do not expect to encounter persisting pain that would lead to the removal of an animal from the study. Post-operative pain will be managed with buprenorphine and/or Meloxicam as described in section J. In the unexpected case that an animal presents signs of pain for 24 or more hours after surgery or during periods of time that are not related to postsurgical recovery, we will consult LARC veterinary staff.

Consultation with a veterinarian will occur if an animal's weight drops 15% below initial surgical weight.

Signs of distress observed during behavioral conditioning experiments will prompt immediate termination of the distressing stimulus or procedure, and a revision of the behavioral protocol if necessary.

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The implanted animals are monitored at least once a week by the investigators to ensure that either infection surrounding the implants or implant rejection can be dealt with appropriately (during training and recording periods the frequency of monitoring can be higher, e.g. 2-5 days per week). Any complications after recovery surgeries will be discussed with the veterinary staff. Infections around the surgical sites will be treated with topical (and possibly systemic) antibiotic(s) after consultation with the veterinary staff. We always consider removal from study and, potentially, euthanasia if such interactions become a hazard to the animal's well-being and health.

Monkeys for which an implanted device becomes a hazard for the animal's health or well-being will be euthanized after consulting with a staff veterinarian. Primates whose general health condition is poor and deteriorating will be euthanized if necessary after consulting a staff veterinarian. Any unforeseen complications (e.g., infection unresponsive to treatment) that result in sustained lethargy, loss of appetite, or poor grooming unresponsive to intervention by the veterinarian staff will result in euthanasia.

In case of respiratory suppression (e.g., following Buprenorphine or Barbiturate administration) a neonatal resuscitation circuit will be available to support ventilation. In the event of apnea, veterinary consultation will be requested to develop a course of action.

J.5

Describe any experimental endpoints that would result in removal of an animal from study. For all investigators housing animals with tumor formation, skin lesions, neurological deficits, or Category E studies, list the expected endpoints of the animal model and the criteria for euthanasia.

K. Species Locations

K.1

Monkey, Squirrel - "LARC Space" Locations

Use LARC Space for Animal Housing of this species.
Use LARC Space for Survival Surgery on this species.

K.3

Monkey, Squirrel - "Non-LARC Space" Locations

Location Redaction Code	Proposed Use	Space Owner
15MI (legacy code: LCO1)	Non-Surgical Procedures	PI Space
15MJ (legacy code: LCNO)	Non-Survival Surgery	PI Space
15MJ (legacy code: LCNP)	Extended Study	PI Space
15N8 (legacy code: LCO0)	Non-Surgical Procedures	PI Space

K.3.1

Monkey, Squirrel - Non-Surgical Procedures - LCO1

Specify the Non-Surgical Procedures conducted at this location:	Behavioral Experiments
Justify the need to use non-LARC space for Behavioral Experiments:	Behavioral experiments are conducted in a large sound attenuation chamber that can't be replicated in LARC facilities.
I have posted a copy of the cleaning SOP in this location:	Yes
Is ABSL2 work conducted in this room:	No

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K.3.1	
Monkey, Squirrel - Non-Survival Surgery - LCNO	
Specify the Non-Survival Surgery procedures conducted in this location:	Craniotomy, electrode placement and electrophysiological recording
Justify the need to use non-LARC space for these non-survival surgeries:	Our experiments and surgeries occur inside of an Anaechoic chamber. We have specialized equipment installed and this cannot be replicated in LARC space
Is ABSL2 work conducted in this room:	No

K.3.1	
Monkey, Squirrel - Extended Study - LCNP	
Will animals be kept in the room for more than 12 hours?	Yes
LARC will provide animal husbandry services:	No
Describe how the laboratory will perform the animal husbandry and care oversight:	Animals are not housed in this space. They might have experimental procedures extending to 12 hours but most animals undergoing procedures in this room are in acute experiments.
Special attention is required for the strain or model:	No
Special post-procedural care is required:	No
Special environmental needs are required (light, temperature, sound, etc.):	No
Hazardous substance containment (Radiation, chemical or biosafety containment) is required:	No
Chronic preparation of animal is required (more than 12 hours):	No
Other requirements of the experiment, phenotype or model:	No
Maximum number of animals housed at a given time:	1
Justify this number:	One animal is experimented upon at a time.
Animals are continuously housed:	No
Is ABSL2 work conducted in this room:	No
Other relevant details:	

K.3.1	
Monkey, Squirrel - Non-Surgical Procedures - LCO0	
Specify the Non-Surgical Procedures conducted at this location:	Behavioral Experiments Cleaning head post implant margins, shaving the hair around the implant to keep it clean, cylinder maintenance to include exposure of the cortex for neurological recordings.
Justify the need to use non-LARC space for Behavioral Experiments:	Behavioral experiments occur in an anechoic chamber with specialized equipment that is not available in LARC space

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I have posted a copy of the cleaning SOP in this location:	Yes
Justify the need to use non-LARC space for each procedure described under 'Other':	Our experiments and surgeries occur inside of an anechoic chamber. We have specialized equipment installed and this cannot be replicated in LARC space
Is ABSL2 work conducted in this room:	No

K.5
Monkey, Squirrel - "Transporting"
You will NOT be transporting animals in your own vehicles.

L. Reportable Exceptions for Procedures

Squirrel Monkey
L.1

M. Physical Restraint of Conscious Animals

M.1	
Will you be physically restraining any animals:	**Yes**

Squirrel Monkey	
M.2	
How many animals of this species will be restrained:	All implanted animals; 6-7 per year
Describe the types of restraint device(s) that will be used:	Squirrel monkeys are seated in a custom designed chair that applies movement restraint with loosely fitted restraints around the neck and waist. The restraints restrict posture to a comfortable seated position. The animal is able to adjust its body posture and to move its arms and legs when seated in the chair. During electrophysiological recording, head position is fixed in a comfortable position by a metal bar that connects the head-mounted metal post to the frame of the primate chair.
For each restraint device, describe the duration and frequency of confinement:	Restraint will be applied for 1-5 hours, up to five days per week during behavioral or behavioral/electrophysiological experiments.
Describe how each animal will be acclimated to each restraint device:	Squirrel monkeys scheduled for experimentation in the chronic recording/behavioral laboratory are slowly adapted to a primate chair that restricts body movements and fixes the head in a position that is comfortable and safe for the animal. These restraints guarantee stabile conditions for microelectrode recording from the brain, and represent the absolute minimum restraint necessary to record from the awake squirrel monkey.
Describe how animals will be monitored and frequency of monitoring during periods of confinement:	Restrained squirrel monkeys are monitored by an investigator via continuous video feedback to the adjacent room in which the investigator is located. Notebook charting of main parameters potentially indicating distress every 60-90 minutes.
Describe the criteria for removal of animals that do not adapt or acclimate to the restraint device:	General behavior, appearance, activity level, vocalizations, and posture will be observed to assess possible distress.

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Describe your provision of veterinary care for animals that may experience adverse clinical consequences:	Animals that experience problems with restraint will be immediately removed from restraint and a veterinarian will be consulted.

N. Euthanasia

Monkey, Squirrel	
N.1	
Will you conform to the UCSF Guidelines for Euthanasia?	Yes

N.2	
Will you ever perform a physical method without a prior chemical method on animals >6 days of age?	No

N.3		
150 - 200 mg/kg sodium pentobarbital IV (primates)	Bilateral thoracotomy	Monkeys will be euthanized with 150 - 200 mg/kg sodium pentobarbital IV (primates) and bilateral thoracotomy.
Perfusion under general anesthesia	Monkeys will be euthanized with 150 - 200 mg/kg sodium pentobarbital IV (primates) and bilateral thoracotomy.	In animals in which perfusion will be performed, the 150 - 200 mg/kg dose of pentobarbital will be followed by chest opening under areflexic anesthesia, and perfusion with phosphate buffered saline followed by fixative solution. The brain will be removed. The dose range is approximate, as the drug is administered to effect and prior doses of pentobarbital require a lower dose to have the animal anesthetized but alive for the start of perfusion.

O. Environmental Enrichment

Monkey, Squirrel	
O.1	
Will you conform to the UCSF Guidelines for Environmental Enrichment?	Yes

O.2	
Will your animals be socially housed?	Yes

P. Tissue Sharing and Live Animal Disposition

P.1						
Monkey, Squirrel - Available Tissues						
Strain	Gender	Age	Weight	Tissues Not Available	Form of Euthanasia	Tissue Alterations

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	Both	Young adult	600-800 grams	Brain and Cochleae	Pentobarbital and thoracotomy	

Monkey, Squirrel	
P.2	
Name and telephone number of the contact person to discuss tissue-sharing arrangements: Name Redacted	
Contact Name:	Number Redacted
Contact Telephone:	[redacted]
P.3	
Live Animal Disposition	
Describe your plan, if you are willing to make live animals available after your study:	

Q. Roles & Training

Name Redacted, Redaction Code: DILN - Details	
Admin Role :	
MHS Questionnaire :	Completed on 02/19/2020
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course NOT Required; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: DILN - Training Records	
Course Title	**Date**
COVID-19 and Working Onsite at UCSF	05/28/2020
IACUC - BRER I Tutorial (Online)	02/19/2020
IACUC - BRER II Tutorial (Online)	02/19/2020
IACUC - Mouse Euthanasia Training	02/24/2020
IACUC - Squirrel Monkey Basic	03/12/2020
Neuroscience Facility Orientation	03/12/2020
UCSF Rodent Barrier Training (Online)	02/19/2020

Name Redacted, Redaction Code: PIB - Details	
Admin Role :	Alternate Responsible
MHS Questionnaire :	Completed on 07/29/2019
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course NOT Required; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: PIB - Training Records	
Course Title	**Date**
Animal Biosafety Level I (Online)	03/27/2008
Animal Biosafety Level II (Online)	03/17/2009

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AWAP - Basic Regulatory and Ethical Req	01/29/2002
AWAP - Rat: Basic Intro	02/14/2002
Biosafety Training	02/01/2019
Biosafety Training (was BSL Level II)	03/12/2009
Bloodborne Pathogens	04/18/2019
Carcinogen Training	04/22/2020
Controlled Substances Training	11/13/2017
COVID-19 and Working Onsite at UCSF	06/01/2020
Emergency Response Awareness Haz-Com	10/28/2005
Formaldehyde Online Training	02/14/2018
Hazardous Waste Management (Online)	04/15/2003
Herpes B Training (Online)	10/01/2019
IACUC - AAALAC Town Hall	08/20/2018
IACUC - BRER I (Online)	11/05/2010
IACUC - BRER I Test-Out (Online)	11/12/2019
IACUC - BRER I Tutorial (Online)	11/12/2019
IACUC - BRER II (Online)	07/02/2008
IACUC - BRER II Test-Out (Online)	11/12/2019
IACUC - BRER II Tutorial (Online)	11/12/2019
IACUC - Herpes B Training	10/01/2015
IACUC - Herpes B Training	10/01/2015
IACUC - Mandated Training	12/24/2008
IACUC - Medical Record Documentation	10/17/2005
IACUC - Mouse: Basic Intro	04/06/2016
IACUC - Mouse: Basic Intro	03/22/2005
IACUC - New World Primates: Basic Intro	09/10/2012
IACUC -- Post Approval Surgery Review	04/03/2014
IACUC - Rat Euthanasia Training	07/08/2010
IACUC - Researcher Care Health Assessment Training	06/17/2015
Lab Safety for Researchers	07/03/2019
Lab Safety for Researchers (Online)	03/28/2007
Lab Safety Refresher w/Fume Hood 2008	03/12/2009
Laboratory Safety for Researchers (2013)	08/16/2013
Neuroscience Bldg 19A Facility Orientation	09/12/2012
PSB Classroom Presentation	02/18/2005
PSB Facility Orientation	02/24/2005
Safe Shipper - Biohazards (Online)	09/16/2011
Safety and Security Awareness for Researchers	02/06/2020
UCSF OEH&S Office Ergonomics (Online)	05/29/2008
UCSF Rodent Barrier Training (Online)	01/11/2010

Name Redacted, Redaction Code: 6JCA - Details	
Admin Role :	
MHS Questionnaire :	Completed on 08/06/2019
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.

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BRER II Training :	Course Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: 6JCA - Training Records	
Course Title	**Date**
Biosafety Training	12/01/2017
Bloodborne Pathogens	04/18/2019
Carcinogen Training	03/06/2020
Controlled Substances Training	08/08/2019
Formaldehyde Online Training	07/25/2018
Herpes B Training (Online)	03/27/2019
IACUC - AAALAC Town Hall	08/20/2018
IACUC - BRER I Tutorial (Online)	06/14/2018
IACUC - BRER II Tutorial (Online)	06/15/2018
IACUC - Herpes B Training	10/30/2015
IACUC - Mouse: Basic Intro	03/15/2017
IACUC - New World Primates: Basic Intro	02/04/2014
IACUC -- Post Approval Surgery Review	05/21/2018
IACUC Mouse Cage Density Policy Training	05/23/2017
Lab Safety for Researchers	07/09/2018
Laser Safety	08/08/2019
Safety and Security Awareness for Researchers	03/06/2020
UCSF Rodent Barrier Training (Online)	03/17/2017

Name Redacted, Redaction Code: E6023 - Details	
Admin Role :	
MHS Questionnaire :	Completed on 08/04/2019
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: E6023 - Training Records	
Course Title	**Date**
Animal Biosafety Level I (Online)	04/15/2008
Animal Biosafety Level II (Online)	04/15/2008
Animal Biosafety Level III (Online)	04/15/2008
AWAP - Cat: Exempt	12/31/1998
AWAP - Focused Protocol Audit and Review	08/18/2003
AWAP - Nonhuman Primate: Basic Intro	01/15/2003
Biosafety Training	11/07/2019
Bloodborne Pathogen	05/07/2013
Bloodborne Pathogens	11/26/2017
Controlled Substances Training	10/02/2009
COVID-19 and Working Onsite at UCSF	05/28/2020
Hazardous Waste Management (Online)	05/24/2007
Herpes B Training (Online)	08/02/2015

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IACUC - AAALAC Town Hall	08/29/2018
IACUC - BRER I Test-Out (Online)	04/15/2013
IACUC - BRER I Tutorial (Online)	03/19/2019
IACUC - BRER II (Online)	04/19/2010
IACUC - BRER II Test-Out (Online)	02/16/2019
IACUC - BRER II Tutorial (Online)	02/16/2019
IACUC Certification Level 1 - Surgery	06/01/2003
Lab Safety for Researchers	06/06/2019
Lab Safety Refresher w/Fume Hood 2008	10/02/2009
Laboratory Safety for Researchers (2013)	08/02/2013
Protecting Human Research Subjects	01/08/2003

Name Redacted, Redaction Code: B0OD - Details	
Admin Role :	
MHS Questionnaire :	Completed on 07/18/2019
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: B0OD - Training Records	
Course Title	**Date**
Carcinogen Training	04/22/2020
COVID-19 and Working Onsite at UCSF	05/28/2020
Herpes B Training (In-person), No NHP Contact	07/19/2017
Herpes B Training (In-person), No NHP Contact	07/19/2017
Herpes B Training (In-person), No NHP Contact	07/19/2017
Herpes B Training (Online)	10/30/2018
IACUC - BRER I Tutorial (Online)	07/13/2017
IACUC - BRER II Test-Out (Online)	07/13/2017
IACUC - BRER II Tutorial (Online)	07/13/2017
IACUC - New World Primates: Basic Intro	07/28/2017

Name Redacted, Redaction Code: BJJH - Details	
Admin Role :	
MHS Questionnaire :	Completed on 09/19/2019
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course NOT Required; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: BJJH - Training Records	
Course Title	**Date**
Biosafety Training	01/05/2019
Bloodborne Pathogens	12/06/2019
Carcinogen Training	01/05/2019
Controlled Substances Training	01/05/2019

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COVID-19 and Working Onsite at UCSF	06/01/2020
Ergo: Laboratory Training (Online)	01/05/2019
Formaldehyde Online Training	10/05/2018
IACUC - BRER I Tutorial (Online)	09/23/2018
IACUC - BRER II Tutorial (Online)	09/24/2018
IACUC - Mouse Euthanasia Training	09/26/2018
IACUC - New World Primates: Basic Intro	09/26/2019
IACUC - Rat: Basic Intro	04/11/2019
IACUC - Squirrel Monkey Basic	09/26/2019
IACUC Mouse Cage Density Policy Training	09/24/2018
Lab Safety for Researchers	01/05/2019
Laser Safety	02/17/2019
Neuroscience Facility Orientation	09/26/2019
UCSF Rodent Barrier Training (Online)	09/24/2018

Name Redacted, Redaction Code: 32H2 - Details	
Admin Role :	
MHS Questionnaire :	NOT Completed
BRER I Training :	Course NOT Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course NOT Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: 32H2 - Training Records	
Course Title	**Date**

Name Redacted, Redaction Code: 3BO0 - Details	
Admin Role :	Alternate Responsible,Authorized Purchaser,Correspondence To,Emergency Contact 2
MHS Questionnaire :	Completed on 04/28/2020
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: 3BO0 - Training Records	
Course Title	**Date**
Animal Allergy	05/15/2018
Animal Allergy	05/15/2018
AWAP - Mouse: exempt	12/31/1998
Bloodborne Pathogens	02/20/2008
Carcinogen Training	04/22/2020
Controlled Substances Training	09/25/2018
EH&S Training for IACUC Member and Staff	10/29/2019
Fire Safety (Online)	02/20/2008
Hazardous Waste Management (Online)	02/20/2008
Herpes B Training (Online)	12/05/2019
IACUC - AAALAC Town Hall	08/23/2018

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IACUC - BRER I Test-Out (Online)	04/28/2020
IACUC - BRER I Tutorial (Online)	04/28/2020
IACUC - BRER II (Online)	03/30/2011
IACUC - BRER II Test-Out (Online)	04/28/2020
IACUC - BRER II Tutorial (Online)	04/28/2020
IACUC - Cat: Basic Intro	03/05/2008
IACUC - Faculty USDA Compliance Review	10/23/2017
IACUC - Herpes B Training	10/30/2015
IACUC - Mouse: Basic Intro	03/15/2017
IACUC - New World Primates: Basic Intro	03/17/2008
IACUC - Rat Euthanasia Training	01/29/2013
IACUC- Basics of IACUC Membership	03/21/2017
IACUC Mouse Cage Density Policy Training	03/06/2017
Lab Safety for Researchers	01/04/2017
Lab Safety for Researchers (Online)	02/20/2008
Lab Safety Refresher w/Fume Hood 2008	03/23/2010
Neuroscience Bldg 19A Facility Orientation	06/22/2012
UCSF Rodent Barrier Training (Online)	03/06/2017

Name Redacted, Redaction Code: F6037 - Details	
Admin Role :	Principal Investigator,Emergency Contact 1
MHS Questionnaire :	Completed on 02/05/2020
BRER I Training :	Course Taken; BRER I Test Out option or BRER I Tutorial is required every 3 years for all personnel on IACUC protocols.
BRER II Training :	Course Taken; BRER II is required every 3 years for all personnel whose functional role includes Anesthesia, Surgery and/or Post-Surgical Care.

Name Redacted, Redaction Code: F6037 - Training Records	
Course Title	**Date**
Animal Biosafety Level I (Online)	04/01/2008
Animal Biosafety Level II (Online)	04/04/2008
Animal Biosafety Level III (Online)	04/04/2008
AWAP - Focused Protocol Audit and Review	08/18/2003
AWAP - Focused Protocol Audit and Review	03/17/2003
AWAP - Nonhuman Primate: Basic Intro	01/15/2003
AWAP - Rat: Exempt	12/31/1998
Biosafety Training	02/26/2020
Biosafety Training (was BSL Level II)	08/29/2008
Bloodborne Pathogens	04/08/2010
Controlled Substances Training	08/28/2017
Emergency Response Awareness Haz-Com	10/07/2005
IACUC - AAALAC Town Hall	08/23/2018
IACUC - BRER I Test-Out (Online)	01/25/2013
IACUC - BRER I Tutorial (Online)	01/29/2019
IACUC - BRER II (Online)	01/25/2010
IACUC - BRER II Tutorial (Online)	02/26/2019
IACUC - Faculty USDA Compliance Review	10/23/2017

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IACUC - Mouse Euthanasia Training	09/24/2014
IACUC - Rat Euthanasia Training	01/29/2013
IACUC - Researcher Care Health Assessment Training	06/17/2015
IACUC Certification Level 1 - Surgery	06/01/2003
Lab Safety for Researchers	11/18/2019
Lab Safety Refresher w/Fume Hood 2008	10/20/2009
Laboratory Housing (Online)	02/07/2005
Protecting Human Research Subjects	05/02/2003

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