University of California, Los Angeles
Chancellor's Animal Research Committee (ARC)

Continuation Application

General Information	
Title:	[redacted]
Protocol #:	[redacted]
PI:	[redacted]
Status:	APPROVED_WITH_CODICIL
Approval Period:	1/11/2019-1/10/2020
Received Date:	12/20/2018
Type:	Continuation
Species:	36 Dog (Pain Category D)
Create Date:	12/18/2018 2:51:58 PM
Created By:	[redacted]
Owner:	[redacted]

Updated Sections

Contacts
Continuation Summary
Number of Animals Used
PI Assurance
Pre-Review
Response to Pre-Review

Personnel Certifications Due:

[redacted]
• MHQ (val d until 1/8/2020)

[redacted]
• MHQ (expired on 10/29/2019)

Notes:

• General Certif cation Test: Offered through CITI program (**http://www.citiprogram.org**). Please ensure your affiliat on is listed as UCLA and complete the Animal Research Basic Course.
• Medical History Questionnaire (MHQ): Offered by the Occupat onal Health Facil ty (**http://mhq.healthsciences.ucla.edu/**).
• Species Specific Training: Please visit the DLAM webs te: **https://portal.dlam2.ucla.edu/EducationTraining/Pages/default.aspx**.
For more questions regarding certifications/training, please visit:
http://ora.research.ucla.edu/rsawa/arc/pages/certification_info.aspx.

Codicil(s):

• The Committee understands that you will contact a DLAM veterinarian prior to conducting surg cal procedures in [redacted] so that the animal's response may be evaluated. You may contact Dr. [redacted] to schedule this.

Continuation Summary

Please provide the appropriate information regarding changes to this protocol. Then update the respective sections.

1. **Will you be planning on making changes to this protocol? If you answer "yes" to this question, please address Questions 2 and 3 and update the appropriate sections that are affected by these changes. Please note that you are not required to update every section of your protocol.**

   ○ Yes ◉ No

2. **Check the following if you will be making any of the following changes:**

   In addition to checking these boxes, you must update the respective sections of this protocol.

   ☐ Protocol title
   ☐ Funding or funding agency
   ☐ Principal investigator
   ☐ Co-investigator
   ☐ Personnel
   ☐ Location

3. **Indicate if you will be making any Significant changes to the following:**

   In addition to checking these boxes, you must update the respective sections of this protocol.

   ☐ Animal species and/or strain
   ☐ Number of animals
   ☐ Pain category
   ☐ Method of euthanasia
   ☐ Experimental procedures

A. If you indicated that you will be changing the number of animals above, please provide a detailed explanation of your rationale for the number of additional animals requested. Please note that if this request for additional animals also entails a change in experimental procedures and/or pain category, please update these application sections and indicate these changes on this page.

B. If you indicated that you will be changing the experimental procedures above, please provide a detailed explanation of how this change in experimental procedures relates to the experiments in your currently approved protocol. In addition, please clarify what results you hope to yield from this change in experimental procedures.

4. **In order to assist reviewers, briefly describe in lay terms the changes you are making and complete the appropriate sections.**

5. **To assist the ARC in documenting scientific progress arising from use of animals under this protocol, please provide ONE of the following:**

- ○ Citation(s) of presentations or articles resulting from this protocol (either accepted or submitted). Please include an abstract.
- ◉ A brief (1-2 sentence) update regarding progress made toward achieving the scientific objective(s) of this protocol.
- ○ A copy of the most recent annual progress report submitted to the funding agency.

If the scientific progress documentation is in a text format, please paste (or type) it here. Otherwise, you will need to submit it to the ARC as a hard copy.

Major Activities
Over the past year, the major activity has been to refine our procedures prior to initiation of experiments.

Specific Objectives
The four specific objectives of this research are to investigate the following questions:

1. Based on highspeed endoscopic imaging from full larynx experiments, and highspeed imaging of the medial surface vibrations of the vocal folds from hemilarynx experiments, how do prephonatory glottal posturing and vocal fold dynamics change as a function of changes in the activation levels of the CT, TA, LCA+IA, and PCA muscles, both with and without a vocal tract? As a function of neuromuscular input to these muscles, are vocal fold vibrations more appropriately characterized as being vocal tract dependent or independent? Also, as reported by [redacted] are vocal register changes always accompanied by corresponding changes in laryngeal vibratory mechanisms, even when frequency jumps do not occur and no consistent, reliable perceptual detection of the register change is made? For each distinct vocal register or vocal fold vibratory pattern which is identified, what unique laryngeal vibratory mechanisms are suggested?
2. Based on auditory perceptual testing, what changes in voice quality are detected as a function systematic changes in the activation levels of the CT, TA, LCA+IA and PCA muscles, both with and without a vocal tract? As a function of neuromuscular input to these muscles, are such perceived differences in vocal quality more appropriately characterized as being vocal tract dependent or independent? Many analysis-by-synthesis studies in voice quality---in which inverse filtering is an integral component of the technique---implicitly assume that the source and tract are independent. But how valid is this assumption across a wide range of neuromuscular conditions? Moreover, are these voice quality ratings spread fairly evenly along a continuum, or do the voice quality ratings cluster in distinct, non-overlapping perceptual regions indicative of changes in vocal register? And if such clusters do exist, how do these perceptually-categorized clusters correspond to any vocal register changes identified by the laryngeal vibratory mechanisms (e.g., see Specific Aim 1)?
3. What changes in phonation threshold pressure, vocal efficiency, and tissue collision dynamics occur as a function of changes in the activation levels of the CT, TA, LCA+IA and PCA muscles, both with and without a vocal tract? How do these changes correspond to changes in laryngeal vibratory mechanisms, and perception of voice quality and vocal register?
4. Based on the medial surface dynamics from the hemilarynx experiments, and a 3D computational model, what is the optimized biomechanical stiffness of the body and cover layers of the vocal folds as a function of systematic changes in the activation levels of the CT, TA, LCA+IA and PCA muscles, both with and without a vocal tract? And how do these stiffness changes in the body-cover layers of the vocal folds correspond to changes in laryngeal vibratory mechanisms, phonation threshold pressure, vocal efficiency, tissue collision dynamics, and perception of voice quality and vocal register, as studied in the foregoing aims?

Key Outcomes or Other Achievements
The major achievement for this year has been refinement of our experiment procedures prior to initiation of experiments.

6. **Please indicate whether any adverse effects or unanticipated problems have been experienced, including higher than anticipated mortality/morbidity regardless of the cause. If so, please provide an explanation of how these events/problems were resolved.**

No

7. **Please respond to the following questions regarding alternatives to the use of animals. If you answer YES to any of these items, please explain.**

A. Have any alternatives become available since the previous ARC approval that could replace the use of animals to achieve your research and/or teaching goals?

No

B. In order to reduce potential pain/distress, have any procedural refinements been made since the previous ARC approval?

No

C. Has the number of animals required for the study been reduced since the previous ARC approval?

No

Number of Animals Used

List the species and the total number of animals used in the previous year.

Species	Total Number Used
Dog	0

Research Summary

Your answers to the questions on this page determine the other sections needed to be filled out.

1. What is the Title of the Project?

[redacted]

2. Check all that apply:

- ☐ Tumor Formation (spontaneous or implanted)
- ☐ Chronic Disease (diabetes, EAE, status epilepticus, etc.)
- ☑ Tissue Collection (blood and all other tissues, including those collected after euthanasia)
- ☐ Antibody/Ascites Production
- ☑ Surgical Procedures (survival, non-survival)
- ☐ Non Surgical Procedures (injection of experimental drugs, behavioral studies)
- ☑ Gas Anesthetic Agent(s) (use of isoflurane, halothane, etc)
- ☐ Hazardous Agents (carcinogens, paraformaldehyde, rDNA, vectors, etc.)
- ☐ Radioisotopes or radioactive implants
- ☐ Prolonged Physical Restraint (physical restraint of unanesthetized animals for periods longer than 15 minutes)
- ☐ Genetically Modified Animals
- ☐ Tissue Sharing (use of tissues only)

3. Will the research be conducted exclusively on tissue received from another investigator?

No

If yes, do your funding sources require an ARC approved protocol?

No

4. Check all that apply:

- ☐ Experiments done entirely at another institution

  NOTE: For experiments conducted entirely at another institut on please subm t the most recent approval not ce and a copy of the most recently approved protocol from the other institution with your submission. Please also indicate the PHS Assurance number and AAALAC accreditation status.

- ☐ Experiments done entirely at VAGLAHS
- ☐ Program Project/Training Grant

  Administrative approval only – no animals associated with this protocol.

- ☐ Breeding Colony: #

  NOTE: If you will be breeding animals for this protocol and do not already have an approved breeding protocol on file w th the ARC, you must submit an Appl cat on to Establish and/or Maintain a Breeding Colony at this time. Check the box above but leave the "Breeding Colony Number" field above empty. The ARC Staff will update the Breeding Colony Number following the submission of a breeding colony application.

5. If you are seeking approval for a training grant, list all individual projects supported by the program project or training grant, including the principal investigators' names and their current ARC approval numbers. If no animal research is currently being supported by the overall grant, please assure the Committee that, should an investigator of a project covered by the overall grant initiate research involving animals, ARC approval will be obtained prior to the distribution of funds.

Personnel

There can be only one Principal Investigator per protocol. To edit a person's contact information or add a new person to our system, click on the People tab above.

Prior to the submission of an amendment to add personnel, please ensure that these individuals have completed all applicable animal use certification requirements and have a Medical History Questionnaire (MHQ) on file with the Occupation Health Facility (OHF). If you are only requesting the removal of personnel, please email the ARC administrative office (arc@research.ucla.edu). An amendment application is NOT required if you are only removing personnel.

Principal Investigator

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:		Dept:	[redacted]
Status:	Faculty		

What role will this person be performing in this protocol?

Principal Investigator

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

No

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] has worked with the canine laryngeal model since [redacted] is an international expert in the procedures of [redacted] which will be conducted in this experiment. [redacted] has completed the species-specifc training on canines.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Study design, supervision of experiment, computer control of electronics, imaging and data collection aspects of this proposal.

Animal surgery and handling will be primarily performed by [redacted] and [redacted] However, Dr. [redacted] may assist in some aspects of the experiment where contact and handling of the animal may be necessarily.

Dr. [redacted] will not be directly involved in surgical procedures.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	[redacted]
Fax:	[redacted]	**Dept:**	[redacted]
Status:	Faculty		

What role will this person be performing in this protocol?

Co-Investigator

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

No

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] is a board-certified [redacted] surgeon. [redacted] has worked in the [redacted] Lab using the in vivo canine model since [redacted] is a [redacted] with clinical and research endeavors in voice disorders.

Dr. [redacted] has experience with all aspects of this protocol, including canine anesthesia, surgery, neurmuscular stimulation, intralaryngeal dissection of laryngeal nerves, and in vivo phonation. [redacted] has completed the species-specifc training on canines.

For the past [redacted] years [redacted] has been the co-director of the [redacted]

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

Animal surgery, intralaryngeal dissection, neuromuscular stimulation, high-speed imaging, recording of experimental parameters.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:		Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Dog

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

No

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] has worked with the canine laryngeal model in the Department [redacted] Labo tory since [redacted] Since [redacted] has also worked in the Department of [redacted] Laboratory. [redacted] has previously performed all surgical aspects of this protocol including intra-laryngeal micro-dissection. [redacted] is also the caretaker of the [redacted] Laboratory.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

[redacted] will assist with all animal handling and surgical aspects of protocol.

Will this person handle radioactive materials or radioactive animals?

No

Contacts

Name:	[redacted]
Contact Type:	Administrative
Home Phone:	[redacted]
Mobile Phone:	[redacted]
Email:	[redacted]

Name:	[redacted]
Contact Type:	Emergency, Administrative
Home Phone:	[redacted]
Mobile Phone:	[redacted]
Email:	[redacted]

Funding

1. Funding Types (Check All That Apply):

- ☐ Department
- ☑ Extramural
- ☐ UCLA Academic Senate
- ☐ Gift
- ☐ No funding at this time
- ☐ Other:

Proposals

List all funding agencies to which this animal protocol has been or will be submitted for consideration. Include all pending applications.

For each grant/proposal subm tted to a funding agency, subm t a copy of the grant proposal. If the agency is not listed, please contact the **Office of Contracts and Grants.** Please note that the National Institutes of Health may be found by typing in the keyword "NIH" when searching for an Agency Code.

Please note that the Publ c Health Service (PHS) Policy requires the Inst tution to verify approval of those components of the grant applicat on or proposal related to the care and use of animals. **Therefore, it is strongly recommended that prior to submission, investigators review all of the proposed experiments pertaining to animals in the grant application to ensure congruence with the animal research protocol. Please detail any inconsistencies between the grant and the protocol in the spaces below.**

Agency Name:

[redacted]

Agency Code:

[redacted]

PI of Proposal/Award:

[redacted]

Proposal/Award Title:

[redacted]

Proposal/Award Number:

[redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

None.

Rationale

1. Provide a non-technical summary of the overall objectives of the study.

The primary objective of this research is to better understand how lung pressure, the voice box (i.e., the larynx), and the vocal tract work together to produce transitions in vocal registers in both speech and singing. Using an intact neuromuscular model of the larynx, both with and without a vocal tract, we will implement comprehensive, systematic variations in lung pressure and the activation levels of selected intrinsic muscles of the larynx to document their influence on vocal fold vibration, vocal register, and voice perception.

2. Indicate the possible benefits to mankind and/or animals or the advancement of knowledge that may be derived from this study.

Successful completion of this research will impact current treatment of voice disorders in both speakers and singers, better substantiate the level of interaction between the vocal folds and the acoustic resonances of the vocal tract necessary to produce transitions between vocal registers, and improve our overall understanding of the various vocal registers which can be produced by the larynx (or voice box).

3. Explain the rationale for the use of animals, including (a) why the chosen species is the most appropriate for the study and (b) why the chosen species cannot be replaced with a phylogenetically lower species. Note that cost cannot be accepted as a justification.

For ethical reasons, these invasive studies could not be performed on an in vivo human larynx. However, the canine larynx closely resembles the human larynx in neuromuscular anatomy, overall dimensions of the vocal fold, and microanatomy. While efforts have been made to develop a Frankenstein-like, living, ex vivo, perfused human larynx for phonation, currently only a few phonatory conditions have been produced with this method[redacted] Thus, many further refinements are still needed before phonation may be sustained in such a model for the length of time needed for a comprehensive, systematic study, as proposed in this application. Furthermore, the same ex vivo, perfused method applied to the canine larynx does not yet yield a reliability or consistency comparable with the in vivo canine larynx [redacted]

Phylogenetically lower species do not have larynges with dimensions and physiologic properties similar to that of the human larynx. Moreover, the canine larynx is unique in its ability to allow adequate control and measurements for various aspects of laryngeal physiology, especially with regard to the simultaneous contraction, stiffening, and bulging of the medial surface of the vocal fold thyroarytenoid (TA) muscle. It is also the only model in which the ability to dissect and stimulate individual branches of the intrinsic laryngeal muscles has been demonstrated.

Experimental Design & Justification for Requested Number of Animals

1. Provide a two- to four-sentence lay description of the experimental procedures written in language easily understandable to a seventh grade student.

While performing a systematic and comprehensive variation of the activation levels of 4 intrinsic muscles of the voice box, investigate the following: (1) document the distinct vocal registers which occur and distinguish the registers by their vocal fold vibration patterns, acoustic output, and muscle stimulation levels, (2) based on auditory perceptual testing, document how the observed vocal registers differ in voice quality, (3) document how the observed vocal registers differ with respect to vocal efficiency (acoustic output power divided by the acoustic input power provided by the lungs), phonation threshold pressure (the minimum lung pressure required to produce that particular register, which is an objective measure of the observed ease of phonation), and vocal fold collision stress, (4) compute the tissue stresses of the vocal folds for each of the vocal registers.

2. Provide a complete description of: (a) all activities involving the use of research animals; (b) a scientific justification for the total number of animals required to conduct this study. The number of animals justified in this section must match the totals in the Pain Category Assignments. To the extent possible, assign all animals to experimental groups, which can be easily distinguished by the independent variables defining each group (e.g.,drug dosages, time points, controls, etc.). Clearly indicate the number of animals needed per group and explain how group sizes were determined, either(i) by statistical analysis, or (ii) where statistics are not applicable (e.g., teaching labs, feasibility studies, antibody production, etc.), on the basis of other considerations (e.g., student/animal ratio, tissue yield per animal, antigen/animal ratio, prior experience, etc.). If statistical analysis is employed to determine the number of animals required, please specify the statistical method used.

We have proposed that thirty-six (36) mongrel canines, approximately 25 kg or more in weight, be used in this research. Male and female canines will be used interchangeably as there are no known systematic differences between the male and female canine larynx. The age range of the mongrel canines will be 1 to 2 years old, as a one-year dog has generally reached its full weight and growth by this time.

The canine will be premedicated intramuscularly with Buprenex. Over the course of the experiment, ventilation with isofluorane will be administered to prevent reflexive response to corneal stimulation, or other signs of inappropriate level of anesthesia, as monitored by respiratory rate, heart rate, muscular relaxation, positive toe pinch, color of mucous membranes, and pulse oximetry. Intravenous dexamethasone will be given periodically to decrease nerve and vocal fold swelling. Core temperature will be measured with a rectal thermometer and maintained with a warming blanket. Oxygen saturation will be monitored, as well as expired CO2.

After premedication, the animal will be placed supine on an operating table, and will be intubated endotracheally. The anterior neck skin will be shaved, prepped and draped in a sterile fashion. A midline incision will be made to expose the trachea, larynx, the superior laryngeal nerves (SLNs), and the recurrent laryngeal nerves (RLNs). A low tracheostomy will be made and another endotracheal tube will be placed for ventilation. The anesthesia machine ventilation circuit will be switched to this tube and the oral endotracheal tube will be removed. The trachea will be transected just superior to the distal endotracheal tube and an airflow circuit connected to an expansion chamber (lung reservoir) will be attached to the trachea for directing airflow across the vocal folds for phonatory experiments. The air supply will be heated and humidified. A complete pharyngotomy will be made at the level of the thyrohyoid membrane to slightly exteriorize the larynx in the neck for easy access for measurement instruments such as high-speed photography. Individual branches of the RLNs with be dissected as needed for each experimental protocol. Miniature stimulating electrodes will be placed on each nerve branches.

After the full larynx experiments have been completed, hemilarynx experiments will be conducted. For the hemilaryngeal experiments, a vertical partial laryngectomy (removal of one vocal fold and false vocal fold) will be performed to create a hemilarynx [redacted] Indian Ink will be used to provide visual markers on the superior and medial surface of the vocal fold. High-speed cameras (see Resources) with a prism and/or mirrors will be used to provide highspeed, stereoscopic imaging of vocal fold posture (static studies) and/or vibration (dynamic studies).

As enumerated in the Research Strategy, for each phonatory condition explored, we will determine the influence of intrinsic laryngeal parameter on a variety of dynamic, acoustic, biomechanic and perceptual output variables. Our stereoscopic imaging techniques, automated extraction of three-dimensional physical coordinates, spatio-temporal analyses of the data, and automated recording of acoustic and aerodynamic parameters have reached a stage of development such that we are now able to automatically deliver the neural stimulation and concurrently measure the acoustic, aerodynamic, and vibratory parameters in of the larynx.

Using newly developed methods of graded stimulation in our laboratory [redacted] we will perform graded stimulation of the laryngeal muscles. In particular, we will stimulate four independent nerve branches in a graded fashion using tripolar electrodes, as outlined in the research strategy. In each experiment, we will determine the threshold and maximal activation stimulating current for each muscle. The stimulation pulse rate (100-200 Hz) of the applied pulse trains will be set high enough to prevent single twitch muscle reponses. The duration of the pulse train will be kept short enough (1.5 seconds) and adequate time will elapse between stimulation pulse trains (3.5 seconds) to prevent fatiguing of the nerves and the innervated muscles and allow continuous recording of in vivo posture change and phonation for up to 4 hours. Based on the empirically determined activation response function of each intrinsic laryngeal muscle we will use at 3-7 different graded stimulation levels (as specified in the Research Strategy) to evaluate the effect of each intrinsic laryngeal muscle on output variables. Our automated neural stimulation and measurement system will concurrently deliver independent stimulation pulse trains to intrinsic laryngeal muscles then automatically record the measured variables of subglottal acoustics, subglottal pressure, supraglottal acoustics, and high-speed video of vocal fold posture and vibration. The stimulation pulse amplitudes corresponding to graded levels for each intrinsic muscle activation will be applied in a randomized way to reduce the potentially confounding effects of the order of stimulation.

Each 1.5 s neuromuscular stimulation condition will be implemented and imaged with a Phantom highspeed camera. Each recording will start with a static condition and end with approximately a 1s of stationary phonation at one of two subglottal pressure (Ps or Ps+1kPa), with phonation onset designed to occur at or before 0.5 s. The image resolution will be 512 x 512 pixels at a sampling rate of 4000 fps (frames per second). For the hemilarynx experiments, the dynamics of the medial surface vibrations of the vocal fold will be imaged from a medial view using stereoscopic techniques reported previously [redacted] Approximately 30 points along the medial surface of the vocal folds will be marked using either microsutures (as in [redacted] or India ink. These markers will provide an array of specific points along the medial surface of the vocal fold which will be tracked over the vibration cycle. As in previous studies, the 30 markers will be placed in 5 vertical rows with 6 markers per vertical row [redacted] Spacing between vertical rows will be approximately 2 mm and spacing between markers along the vertical row will be approximately 1 mm. For the full larynx, the vocal fold vibrations will be imaged from a superior aspect. These images will be downloaded to a hard drive for later analysis using the 10 GB Cinestream download capability of our highspeed camera.

AD/DA (analog-to-ditigal and digital-to-analog) boards will record analog signals simultaneously with the generation of the stimulation pulse trains at a sampling rate of 125 kHz. The following analog signals will be measured and recorded: (1) the DC or time-averaged subglottal pressure (with a Baratron type 220D pressure transducer), (2) the DC or time-averaged glottal airflow (with a precision mass-flow meter, MKS type 558A), (3) the AC subglottal pressure (with a B&K 4182 probe microphone), and (4) the AC acoustic output pressure (with a 1/2" B&K microphone model no. 4193) measured 5 cm superior to the glottis, at a 45-degree angle from the vertical axis to be situated outside of the glottal airstream, and to avoid blocking the view required for imaging vocal fold vibrations.

For each neuromuscular configuration, changes in voice quality and vocal registers will be quantified, especially in terms of vocal fold dynamics, acoustics, biomechanics and perception. For the full larynx, the superior surface will be imaged. For the hemi-larynx, both medial and superior surface dynamics will be imaged, to further quantify mechanisms of vibration along the medial surface of the vocal folds, which is expected to vary substantially as a function of TA muscle activation due to medial surface bulging, and should shed light on the chest-falsetto register transition, as one example. These full larynx and hemi-larynx experiments will be performed on 12 canines per year in Years 1-3 of this proposal to the animal research committee, for a total 36 canines.

Because comprehensive, systematic data of this type are relatively unique, the information needed for power calculations is not available. For example, sample variances are completely unknown. Given the lack of data necessary for formal power calculations, sample sizes have been selected based on the following considerations. We propose to collect and analyze data from nine (9) canine subjects per experiment. Whether or not this is a large enough pool to capture the desired variance, it is the maximum number of experiments we can realistically perform in this time. If the sample size and/or the normality of the distribution does not allow parametric statistical testing, such as ANOVAs, nonparametric testing will be performed. In either case, these methods should produce a large volume of comprehensive, systematically-gathered data regarding the influence of graded neuromuscular laryngeal stimulation on voice quality, phonation type, vocal registers, and vocal register transitions, in terms of vocal fold dynamics, the stiffness of body-cover tissue layers, acoustics and perception. When more is known about the effects of graded neuromuscular laryngeal stimulation on these variables, formal calculations of statistical power can reasonably be undertaken, but this is not possible at present, due to the limited information available, as argued above.

We appreciate the concerns of the Committee. However, in this case, we believe that performing additional preliminary experiments primarily for the purpose of power calculations would needlessly result in sacrificing additional animals. Our department has considerable experience with the in vivo canine research methodology (approximately [redacted] years experience), and all of our experience to date suggests that 8-10 dogs is a very good estimate of the number of animals needed to obtain the power we wish. Moreover, the number of proposed subjects is consistent with research performed and published by many other laboratories, in which 8-10 larynges are typically used to generalize results for phonation.

Experiments will be conducted sequentially, and should statistical significance between groups be attained prior to using all 9 animals per group, further experiments will not be conducted.

All compounds will be pharmaceutical grade. All animals will be obtained from a commercial vendor.

[redacted]

Pain Category Assignments

NOTE: A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to wh ch that procedure is applied. Examples of potentially painful/distressful procedures include, but are not lim ted to the following: terminal surgery; exuberant inflammation from adjuvants; ocular and skin irritancy testing; food or water deprivation beyond that necessary for normal presurgical preparation; noxious electrical shock that is not immediately escapable; paralysis or immobility in a consc ous animal; extensive irradiation.

Category	Description
C	Momentary or no pain/distress (Examples: injections of non-toxic substances; peripheral blood collections not requiring anesthesia; euthanasia and harvesting of tissue only; observing natural behavior; behavioral testing without signif cant restraint or noxious stimuli.)
D	Pain/distress relieved by use of appropriate anesthetics, analgesics, tranquilizers or by euthanasia (Examples: terminal surgery; survival surgery; retro-orbital blood collection; euthanasia of animals showing signs of more than slight or momentary pain and/or distress.)
E	Pain/distress can not be relieved by use of anesthetics, analgesics, or tranquilizers, as the use of these agents would interfere w th the experimental design (Examples: pain research; toxic ty testing.)

Species:	Dog
Strain or Breed (if applicable):	Mongrel
Average Weight:	25 kg
Sex:	Mixed
Pain Category:	D
Previous Number of Animals Approved:	36
Change in Number of Animals Needed (+/-):	0
Number of Animals Needed for the 3 Year Period:	36

Pain Category

1. If the animals are listed under Pain Category D and/or E, check below all criteria that will be used to assess any potential pain/distress/discomfort in the animals. If applicable, include criteria used to evaluate post-operative pain/discomfort.

- ☐ Restlessness
- ☐ Vocalizing
- ☐ Decreased or impaired mobility
- ☐ Conjunctivitis, corneal edema, photophobia
- ☐ Licking, biting, or guarding a painful area
- ☐ Failure to groom, unkempt appearance
- ☐ Open sores/necrotic skin lesions
- ☐ Loss of appetite
- ☐ Weight loss.
  Percentage weight loss (max allowable 20%):
- ☑ Other: N/A as this is a terminal procedure. Animals will be monitored as described in the Surgery section.

2. If the animals are listed under Pain Category E, please specify the pain/distress/discomfort experienced by animals as a result of the experimental manipulations and provide scientific justification indicating why pain/distress/discomfort-relieving methods will not be employed in this protocol.

NOTE: Procedures that may cause more than momentary or slight pain or distress to the animals must be performed with appropriate sedatives, analgesics or anesthet cs, unless withholding such agents is justified for scientific reasons and will continue for only the necessary per od of time.

Not applicable.

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E. Federal Regulations require that investigators consider alternatives (the 3 Rs - replacement, refinement and reduction) to procedures that may cause more than momentary or slight pain or distress to animals.

3. Consider all the alternatives listed below and explain why each of the following is not an available alternative for the proposed potentially painful/distressful procedure.

A. Replacement of animals with non-animal models (e.g., in vitro procedures, computer model) or a phylogenetically lower species:

Human laryngeal physiology has been modeled with computer models, static geometric shape systems, excised human larynges, excised canine larynges, and human laryngeal non-invasive measurements. Our laboratory has experience with all of these models. Computer models cannot yet accurately recreate the mucosal wave of the intact, self-oscillating vocal folds—much less model the intricacies of vocal fold collision, interactions with the nearfield glottal airflow, and possible acoustical interactions with sub- and supra-glottal systems. Indeed, such computationals models have never been evaluated with direct measurements, such as proposed in this investigation. Excised laryngeal models do not have the same measurable mucosal wave movement that occurs from stimulation of the recurrent laryngeal nerve, nor can they model active muscle contraction, including simultaneous TA stiffening, contraction and bulging. Finally, the proposed research method employs intricate microsurgery of the larynx and invasive control of laryngeal physiological variables that cannot be obtained in human subjects. Therefore, only anaesthetized canine in vivo phonation allows for the control of independent variables and the ability to measure dependent variables invasively. The in vivo canine larynx has dimensions and physiologic properties similar to that of the human larynx.

Phylogenetically lower species do not have larynges with similar properties. Moreover, the canine larynx is unique in its ability to allow adequate control and measurements for various aspects of laryngeal physiology, especially with regard to the simultaneous contraction, stiffening, and bulging of the medial surface of the thyroarytenoid (TA) muscle. It is also the only model in which the ability to dissect and stimulate individual branches to the intrinsic laryngeal muscles has been demonstrated. For these reasons, the canine is the most frequently used in vivo phonatory model, and the most appropriate for this proposed research.
The studies described above represent some for the first attempts to systematically gather a comprehensive set of quantitative vibratory, acoustic and perceptual data regarding the influence of subglottal pressure and neuromuscular stimulation on voice quality and vocal registers. Because comprehensive, systematic data of this type are relative unique, the information needed for power calculations is not available. For example, sample variances are completely unknown.

As also mentioned earlier, our laboratory has experience with all models for investigations of laryngeal physiology: computer models, static geometric shape systems, excised human larynges, excised canine larynges, and human laryngeal non-invasive measurements.

Computer models cannot yet accurately recreate the mucosal wave of the intact, self-oscillating vocal folds—much less model the intricacies of vocal fold collision, interactions with the nearfield glottal airflow, and possible acoustical interactions with sub- and supra-glottal systems. Computer models are based on in vivo experiments; however such in vivo investigations have never been performed. Therefore, this in vivo study is necessary. However, this study will ultimately improve computer models, which will then lead to reduction in the need for animals for exprimentation.

Excised laryngeal models do not have the same measurable mucosal wave movement that occurs from stimulation of the recurrent laryngeal nerve, nor can they model active muscle contraction, including vocal fold stiffening and bulging.

Finally, the proposed research method employs intricate microsurgery of the larynx and invasive control of laryngeal physiological variables that cannot be obtained in human subjects. Therefore, only anesthetized canine in vivo phonation allows for the control of independent variables and the ability to measure dependent variables invasively.

The in vivo canine larynx has dimensions and physiologic properties similar to that of the human larynx. Phylogenetically lower species do not have larynges with similar properties. Moreover, the canine larynx is unique in its ability to allow adequate control and measurements for various aspects of laryngeal physiology, especially with regard to the simultaneous contraction, stiffening, and bulging of the medial surface of the thyroarytenoid (TA) muscle. It is also the only model in which the ability to dissect and stimulate individual branches to the

intrinsic laryngeal muscles has been demonstrated.

For these reasons, the canine is the most frequently used in vivo phonatory model, and the most appropriate for this proposed research.

B. Please discuss why the procedures cannot be further refined in order to minimize potential pain and/or distress to animals:

The experiments will be performed under general anesthesia. We will monitor the vital signs (blood pressure, oxygen saturation, heart rate) closely to ensure adequate levels of anesthesia. We will use a warming blanket and monitor core temperature using a rectal thermometer. Eyes will be taped shut after placement of opthalmic ointment to prevent dessication. At the end of the surgery the animals will be humanely euthanized (note that all surgeries are non-survival surgery). Therefore, we will take all known and necessary measures to minimize potential pain and distress to the animals.

C. Reduction in the number of animals proposed in this application (e.g., fewer animals involved in potentially painful procedures):

All surgery is non-survival, and measures are taken to eliminate pain and distress to the animal during the experiment as the canine will be under general anesthesia throughout the experiment. Nine animals are requested for each experiment, the minimum number needed to demonstrate validity of experimental findings between animals.

Pain Literature Search

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E.

Please note that according to PHS Policy IV.C.1.a, the Guide for the Care and Use of Laboratory Animals (the Guide p. 10) and USDA Animal Welfare Act Regulat ons §2.31(d)(1)(i) "procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals." Further, in order to meet the above-ment oned regulatory requirement and in accordance with UCLA's Animal Welfare Assurance on file w th the National Institutes of Health Off ce of Laboratory Animal Welfare (OLAW), the Committee must ensure that the "principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has prov ded a wr tten narrative descript on of the methods and sources used to determine alternatives were not available." Please also note that the Comm ttee recommends the use of keywords that are specif c to the painful/distressful procedures you will be conducting and the animal model that will be used.

1. Indicate at least two databases or other sources consulted to support the conclusion that appropriate alternatives are not available.

- [x] Pubmed (Medline)
- [ ] PsychINFO
- [ ] Altweb
- [ ] UC Center for Alternatives
- [ ] Animal Welfare Information Center
- [ ] BIOSIS
- [ ] Current Contents
- [x] Other:

ISI Web of Knowledge

2. Combination of keywords used during the search:

Please specify the keywords used in the box below, including 1) the specific painful procedures that you are conducting, 2) the animal model being used and 3) alternative terms (e.g., animal model, welfare, pain, stress, distress, methods, *in vitro*).

Please see the following examples, noting that the keywords listed only apply to a protocol involving these experimental variables:

Mouse and chronic implant and in vitro model
Mouse and artery ligation and pain
Mouse and sleep deprivation and welfare

Keywords used:

SEARCH PERFORMED ON 1/19/2017
Two Databases:
PubMed (Medline)
Other, ISI Web of Knowledge (including all databases: Web of Science, BIOSIS previews, CABI: Global Health, Zoological Record, and Journal Citation Reports)

Keywords Used:

(dog OR canine) AND (larynx OR phonation) AND (in vivo)

102 publications in PubMed
108 publications in Web of Knowledge

Unfortunately, most of these articles are regarding excised human or canine larynges, with no neuromuscular viability. Based on our review of these publications, we believe only the following 3 articles (taken from the above lists) impact the pain literature review of our study:

[redacted]

[redacted]

All of these articles were cited and influenced the design and set-up of our study.

SEARCH PERFORMED ON 1/19/2017
Two Databases:
PubMed (Medline)
Other, ISI Web of Knowledge (including all databases: Web of Science, BIOSIS previews, CABI: Global Health, Zoological Record, and Journal Citation Reports)

Keywords Used:

(dog OR canine) AND vocal fold AND nerve stimulation AND (pain OR distress OR alternatives OR welfare OR in vitro)

** 2 publications in Pub Med. Both were out-dated in vivo canine studies, with no implications for proposed research in terms of alternative models or minimization of pain: [redacted]

** 3 publications in ISI Web of Knowledge. One was a publication already identified in PubMed, plus two others, which did not involve phonation from the dog, but focused solely on swallowing, and did not involve the sophisticated phonatory procedures utilized in our proposal, and contained no additional information regarding minimization of pain in the canine for these procedures. The three publications were: [redacted]

3. Date of most recent search (MM/DD/YYYY):

NOTE: The literature search must be updated whenever experiments that may cause potential pain or distress are proposed/modified. The literature search must also be updated at the time of each three-year renewal, and should be conducted within 2 months of submission.

1/19/2017

4. Years Covered (e.g., 1980-2019):

1966 - present (PubMed). 1864 - present (ISI Web of Knowledge)

Animal Care

1. Will the experiments involve tumor formation?

The ARC requires daily monitoring of tumor growth.

No

2. Will the experiments involve chronic disease (e.g., diabetes, chronic seizures, infections with disease agents) or a chronic condition (e.g. headcaps, implants)?

No

3. Will the experiments involve other procedures that may lead to potential complications (e.g., surgical procedures, administration of compounds with potential toxic effects)?

No

4. For all types of experiments, if animals may experience complications, please describe the criteria for premature euthanasia below.

N/A.

5. Check below all that apply to convey special animal care requirements to the responsible veterinary staff.

- ☐ Temperature Range(s)
- ☐ Humidity
- ☐ Light Cycles
- ☐ Bedding/Litter changing schedules
- ☐ Water (e.g., sterile or deionized)
- ☐ Special diet/Feeding schedule
- ☐ Deprivation of food and/or water for reasons other than surgical preparation

6. If you checked any of the boxes above, explain special care requirements in detail.

N/A

7. Environmental Enrichment: UCLA vivarium staff provide environmental enrichment to all species (please refer to the **ARC Policy on Environmental Enrichment**).

a. If you request to provide additional or alternative environmental enrichment, please describe the environmental enrichment below.

N/A

b. Please provide scientific justification if your research precludes the use of environmental enrichment.

Pair housing is acceptable whenever possible.

8. If you will be using transgenic animals in this research, please clarify whether there are any anticipated or suspected phenotypes of the transgenic mice that might cause pain or discomfort to the animals. If any pain, distress, or morbidity is associated with the phenotypes of this line, please indicate the criteria for premature termination of these mice.

N/A

9. PLEASE COMPLETE IF YOU HAVE MICE AND/OR RATS IN DLAM-MANAGED FACILITIES. Please check one response to the following:

I request that the veterinarian (or his/her designee) euthanize animals found to be sick or injured for me:

○ I request that the DLAM veterinarian (or his/her designee) euthanize my animals for me in accordance with his/her veterinary discretion at the time that they are found sick or injured. This decision will only apply to animals in cages that I've marked with a green euthanasia sticker on the cage card. DLAM will notify me of the euthanasia by email after the fact.

I understand that I remain responsible for monitoring of my animals, in accordance with my approved protocol and with the ARC Policy on ***Responsibility for Monitoring Laboratory Animals***.

I will treat or euthanize animals:

◉ I assure the ARC that I will promptly respond to Veterinary Health Case notifications regarding my animals, as required by the ARC Policy on ***Notification of Investigators with Sick or Injured Animals***.

Locations

Please indicate ALL locations where animals will be housed and/or used, including:

1. **Vivarium Housing** (where animals will be housed). Please note that if vivarium housing has not been assigned, select "VIVARIUM" as the building name and "Unassigned" as the room number.
2. **Study Area** (any investigator-maintained facility outside the vivarium where USDA-covered species will be housed for per ods longer than 12 hours, or where non-USDA-covered species will be housed for per ods longer than 24 hours).
3. **Research Area** (where non-surg cal activities, including euthanasia, will be performed).
4. **Surgery Area - Survival** (where recovery surgery will be performed).
5. **Surgery Area - Non-Survival** (where terminal surgery will be performed).

Building	Room	Species	Location Type
[redacted]	[redacted]	Dog	Surgery Area - Non-Survival **Reason:** Surgery, neuromuscular stimulation, induced phonation, high-speed photography, acoustic and aerodynamic recording, will be done in this room. Only non-survival surgery is proposed in this protocol. The study area is the [redacted] that permanently houses all the bulky equipment needed for these experiments (high-speed cameras, phonation apparatus, hi-fidel ty recording dev ces). The experimental apparatus is delicate and sensitive, calibrated to the study area, and cannot be moved to another location. Because [redacted] was pending ARC approval due to inactivity per a 2/24/2015 wr tten notificat on, I hereby confirm that I will notify [redacted] at [redacted] prior to conducting surgery to arrange an inspect on for this area.
[redacted]	Unassigned	Dog	[redacted]

Medications and Experimental Drugs

List below all medications/drugs/compounds/agents/etc. that will be given to the animals. Please be sure to include analgesics, anesthetics, antibiotics and all experimental drugs or treatments. Cell lines injected in suspension should be listed here.

The select on of the most appropriate med cation/agent should reflect that which best meets clin cal and humane requirements w thout compromising the scientific aspects of the research protocol. In accordance w th federal regulat ons, consultat on with an attending veterinarian is required in the planning of a research protocol involving procedures that may cause more than momentary or slight pain or distress to the animals. The **ARC Policy on Use of Pharmaceutical-Grade Compounds** requires that investigators use pharmaceut cal-grade compounds whenever they are available, even in acute procedures.

If pharmaceut cal-grade preparations are not available, please dentify wh ch compounds are affected and provide supporting justification in your Experimental Design. All non-pharmaceutical-grade drugs must be filter-sterilized pr or to use.

Please do not list euthanasia drugs in this section.

Drug/Compound Name:	Buprenorphine
Species:	Dog
Medication Type:	Analges c
Dose or Concentration:	0.01 - 0.02 mg/kg
Volume:	0.0002 - 0.0004 cc
Frequency:	q6h as needed
Route:	im
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Propofol

Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	6-8 mg/kg
Volume:	0.16 - 0.21 cc
Frequency:	Once for induction of anesthesia
Route:	iv
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Other: Induction of Anesthesia

Drug/Compound Name:	Isoflurane
Species:	Dog
Medication Type:	Anesthet c
Dose or Concentration:	2%
Volume:	250 - 500 mL
Frequency:	Continuous inhalation during surgery
Route:	other: Via tracheal intubation
Length of treatment/administration:	Intraoperative
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Cephazolin
Species:	Dog
Medication Type:	Other
Dose or Concentration:	25 mg/kg
Volume:	3.1 cc
Frequency:	once
Route:	im
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Acepromazine
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.05 mg/kg
Volume:	12.5 cc
Frequency:	Once for premed cat on prior to transport
Route:	im
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Other: Tranquiizer prior to transport

Drug/Compound Name:	Dexamethasone
Species:	Dog
Medication Type:	Other
Dose or Concentration:	0.5 mg/kg
Volume:	0.01 cc
Frequency:	Once after intubation
Route:	iv
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative

Euthanasia

For each species used, please provide the euthanasia information. Techniques for euthanasia must follow guidelines established in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

1. **Species:**

   Dog

2. **How will animals be euthanized?**

   Non-Physical Method

3. **For animals that will be euthanized by a physical method, please indicate that method (decapitation or cervical dislocation).**

   a. **Please indicate the appropriate physical method.**

   Other:Death to be confirmed by absence of heartbeat, respiration & graying of mucus membranes for 10 mins.

b. Will anesthesia be used prior to use of the physical method of euthanasia?

Yes

c. If anesthesia cannot be administered, please provide scientific justification.

4. For animals that will not be euthanized at the end of the study, please indicate the final disposition.

Euthanasia Medications

List the drug(s) used for euthanasia on an animal by physical or non-physical methods.

Please note that according to the **AVMA Guidelines for the Euthanasia of Animals: 2013 Edition**, "compressed CO2 in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable."

Drug Name:	Veterinary Grade Eutha-6 (Pentobarbital)
Species:	Dog
Dose or Concentration:	100 mg/kg
Route:	iv
Purpose of Drug:	Euthanasia

Tissue Collection

Please enter the following information regarding tissue collection for the protocol. See ARC Policy on Blood Collection from Laboratory Animals.

1. Tissue To Be Collected:

- ☐ Blood
- ☑ Other Collected: Larynx, for additional neuroanatomic studies or fur use in excised larynx experiments

2. Frequency of blood and/or other tissue collections:

Once after euthanasia.

3. Volume of blood and/or other tissue collected per time point:

One larynx.

4. Describe techniques that will be used to collect blood and/or other tissue.

The larynx will be removed using standard laryngectomy techniques. It will be extracted with a portion of the trachea attached.

5. Describe how anemia and infection will be prevented.

N/A

Surgical Procedures and Post-Operative Care

Please complete the following questions, noting that any requested exception to ARC Policy must be justified in the space provided.

Note: ARC pol cy requires investigators to employ the following measures to ensure asepsis while conducting survival surgery: asept c surg cal techniques; asept c surg cal field; sterile instruments; clean lab coat/surg cal gown; and sterile surg cal gloves. *For information on surgeries on rodents and birds, please see the **ARC Policy on Survival Surgery in Mice, Rats and Birds***.

Non-survival surgeries of extended durat on or procedures otherwise likely to increase the risk of Intraoperative infection and/or sepsis (e.g. gastrointestinal surgery) will be evaluated on a case-by-case basis to determine whether aseptic techn ques must be used. Refer to the **ARC Policy on Non-survival Surgical Procedures** for further informat on.

Please note that surgical records are required for all animals. These records must include anesthetic administration and intra-operative mon toring, as well as post-operative recovery observations, including administration of analgesics and antib otics and suture/staple removal if appl cable. Additionally, any adverse outcomes must also be recorded.

1. Pre-Operative care will include (check all that apply):

- ☐ Lab tests
- ☐ Conditioning
- ☑ Fasting: Eight (8) hours
- ☐ Other:

Please note that a phys cal examinat on is required.

2. Will neuromuscular blocking agents be used (e.g., Pancuronium, Succinylcholine)? Refer to the ARC Policy on

Neuromuscular Blocking Agents.

No

3. Select all criteria that will be used to assess the proper level of anesthesia.

The level of anesthesia should be assessed on a continuous basis.

- [x] Respiration rate
- [x] Heart rate
- [ ] EEG
- [ ] EKG
- [x] Muscular relaxation
- [x] Positive toe pinch
- [x] Corneal reflex
- [x] Color of mucous membranes
- [x] Other:

Pulse oximetry

4. Surgical preparation of all mammalian species must include:

1) Removal of hair w th #40 clipper blade in a wide margin around the incis on site.
2) Three alternating scrubs using a germ cidal scrub and 70% alcohol.
3) Placement of lubricating ointment into the eyes.
4) Covering the animal except the surgery site w th a sterile drape.
5) Placing the animal on an external heat source (water circulating heat pad or heating pad set on "low" with a barrier placed between the animal and the heating pad).

- (•) I assure the ARC that surgical preparation will be performed as outlined above.
- ( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

PLEASE NOTE: Any deviation from the policies above must be detailed and scientifically justified in the space below.

We already follow all of the items listed above although only non-survival surgery is proposed. In a previous version of a similar protocol, the ARC requested the following and we plan to continue to follow this protocol:

1. Prep the animal and perform the surgery aseptically for the animal preparation and surgical exposure portion of the experiment. The canine will be premedicated intramuscularly with Buprenex and Acepromazine and transported to the laboratory. An intravenous line will be placed in the laboratory and Propofol will be used for induction of anesthesia. After induction, the animal will be placed supine on an operating table, and will be intubated through the mouth with an endotracheal tube. General anesthesia will be maintained with isoflurane (13%), and intravenous propofol as needed, to prevent reflexive response to corneal
stimulation, or other signs of inappropriate level of anesthesia, as monitored by respiratory rate, heart rate, muscular relaxation, positive toe pinch, color of mucous
membranes, and pulse oximetry, etc. Animal preparation and surgical exposure of the larynx involves the first half (about 2-3 hours) of the proposed experiments.

2. For the laryngeal stimulation and data measurement part of the experiments, there are many instruments that cannot be sterilized. For example, the delicate nerve stimulation electrodes, glass prisms used to provide stereoscopic views for the high speed photography, and the subglottal tubes and instrument system used to measure pressure and acoustics cannot be sterilized. These can be cleaned by soaking in alcohol and rinsing under clean water. Sterilizing these equipment places undue burden on the feasibility of this proposal as these instruments are expensive and can break during the sterilization process. For example, each stimulation electrode costs about $1000. Thus use clean conditions.

3. The ARC was concerned about risk of intraoperative infection. However, please be aware that our lab has the most experience in the world using this animal model, and we have been routinely performing similar experiments in the last [redacted] years without development of a single case of intra-operative infection. However, to further allay the concerns of the ARC, I will continue administration of a peri-operative dose of antibiotics and steroids.

i. Cephazolin 1 gram prior to incision.
ii. Dexamethasone prior to incision.
iii. Aseptic animal preparation and surgery (about 3 hours) on the larynx, then clean experimental conditions (new, disposable gloves and cap, and a clean gown will be worn by personnel, all instruments clean) during laryngeal stimulation and data measurement (about 3-4 hours)

5. Indicate the methods to be employed to prevent (a) hypothermia and (b) dehydration (including volume of fluids and route). If this question is not applicable to the proposed surgical procedures, provide a brief explanation.

To prevent hypothermia, the veterinarian recommends the use of water-circulating heading pads over heating lamps and/or electr cal heating pads. The use of heating lamps is strongly discouraged. If not used properly, heating lamps and electrical heating pads may cause thermal injury to the animal. Therefore, describe precaut ons taken to prevent hyperthermia.

(a) A water circulating heading pad is used. Rectal temperature is checked at all times.
(b) Use of lactated ringer or normal saline solution 5 cc/kg/hr IV.u

6. Surgical preparation of the surgeon must include:

1) Wash hands w th germicidal soap.
2) Sterile gloves.
3) Surgical Mask.
4) Cap and booties (not required for mice and rats)
5) Sterile gown (clean lab coat or gown acceptable for m ce and rats)

○ I assure the ARC that surgical preparation will be performed as outlined above.

◉ *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

7. Instrument preparation must be performed by:

1) Autoclave sterilization or ethylene oxide (gas) sterilizat on.
2) Either chemical disinfect on (acceptable between multiple surgeries in mice, rats, and non-mammalian species) or
3) Hot bead sterilizer.

○ I assure the ARC that instrument preparation will be performed using one of the methods outlined above.

◉ *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

8. Duration of Surgical Procedures (Must be completed as applicable):

For non-survival surgery, ind cate the duration from anesthesia induction to euthanasia. For survival surgery, ind cate the durat on from anesthesia induction to recovery from anesthesia.

Survival:	
Non-Survival:	6 - 6.5 hours

9. Provide scientific justification for performing multiple survival surgeries on a single animal.

Multiple survival surgeries will be approved only when they are related components of the experimental design.

N/A

10. Please describe all surgical procedures, including non-survival procedures.

After induction of anesthesia the animal will be intubated endotracheally. Isoflurane general anesthesia is begun. The anterior neck is shaved and prepped with betadine.

A midline vertical neck incision is made. The recurent and superior laryngeal nerves are dissected. A low tracheotomy is made and a new endotracheal tube is placed for ventilation and the oral endotracheal tube is removed. A pharyngotomy is made at the thyrohyoid membrane and the larynx is exteriorized slightly in the neck. The trachea is transected about ring 3-4 to allow infraglottic photography for static experiments, or for airflow for dynamic phonatory experiments. Supraglottic structures of the larynx are removed for improved visualization of the larynx during the experiment.

The individual branches of the recurrent laryngeal nerve are further dissected out. Miniature electrodes are placed for experimental stimulation to activate individual laryngeal muscles.

For hemilaryngeal experiments a vertical partial laryngectomy is performed on one side of the larynx so imaging can be performed of the medial edge of the larynx.

After completion of the experiment, the animal is euthanized. A total laryngectomy is then performed and the larynx collected as described earlier.

11. Please indicate the suture materials to be used:

☐ Internal: absorbable sutures (e.g., Dexon, Vicryl)

☐ External: non-absorbable skin sutures (e.g., Nylon, wound clips). *Please note that external skin sutures or wound clips must be removed 7-14 days following surgery.*

☑ Other/not applicable (describe below):

Non-survival surgery.

12. During recovery from anesthesia, what indications will be monitored to assure the animals are stable?

In accordance with the Guide for the Care and Use of Laboratory Animals, particular attent on should be given to thermo-regulation, card ovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.

N/A

13. How often will animals be monitored after anesthetic recovery?

The ARC requires that animals be observed continuously by trained personnel during the immediate anesthet c-recovery period (i.e., until the animal is ambulatory) and at least daily after anesthetic recovery. However, post-operative mon toring frequency may be greater depending on the complex ty of procedures involved, administration of post-operative analgesia, and the species of animal used.

N/A

Species Surgery

Species:	Dog
Number of Animals:	36
Surgery Type:	Nonsurvival Surgery
Surgeries per Animal:	
Time Between Surgeries:	

Gas Anesthetic

NOTE: Gas anesthetics like isoflurane, halothane, enflurane, and ethane must be used safely. The Off ce of Environment, Health & Safety (EH&S) requires the use of a certified fume hood or a gas anesthet c machine that contains a scavenging dev ce (e.g., anesthet c gas machine w th charcoal filter; ducted fumehood or ducted b osafety cabinet; Crump WAG System; vaporizer w th a scavenging filter, such as F-air canister) when using gas anesthetics.

1. What gas anesthetic agent(s) will be used?

- ☐ Halothane
- ☑ Isoflurane
- ☐ Other:

2. Gas anesthetic(s) will be scavenged via:

- ☐ Certified Fume Hood:
- ☑ Other: Anesthesia Machine - [redacted]

Scavenging Location

This section is empty.

Principal Investigator Assurance

After you have reviewed and answered yes to the items below, please click "Save" at the bottom of the page. Please note that the PI must complete this section. To determine your eligibility to serve as Principal Investigator of a research protocol, please refer to UCLA Policy 900 (Principal Investigator Eligibility) or contact the ARC administrative office (310-206-6308). If the terms of Policy 900 are not met, faculty sponsorship or principal investigatorship by a UCLA employee with faculty appointment may be required.

Regarding policies governing animal research at UCLA:

Yes	No	
◉	○	I agree to abide by all applicable federal, state, and local laws and regulat ons and UCLA pol cies and procedures.
◉	○	I am aware that deviat ons from an approved protocol or violations of applicable pol cies, gu delines, or laws could result in immediate suspension of the protocol.
◉	○	I understand that the attending veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.
◉	○	I declare that all experiments involving live animals will be performed under my supervis on or that of another qualified scientist. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.
◉	○	I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment or illness.
◉	○	I declare that the information provided in this appl cation is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this appl cation accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
◉	○	Any modificat ons to the protocol will be subm tted to and approved by the ARC prior to initiation of such changes.
◉	○	The experimental design has been refined in order to minimize the invasiveness of the proposed procedures.
◉	○	I assure that the proposed research does not unnecessarily duplicate previous experiments.

Agreement on electronic submission:

I understand that by submitting this document that this document will be sent to appropriate members for review. I further understand that once submitted for review, this protocol cannot be modified or changed unless so requested by the ARC. In addition, once approved, all changes or modifications must be subm tted for review and approval of the ARC prior to in tiation.

Completed by: [redacted] **12/18/2018**

FS Assurance

This section is empty.