

2004

APPLICATION FOR APPROVAL TO USE VERTEBRATE ANIMALS IN RESEARCH and TEACHING NEW SUBMISSION

Institutional Animal Care and Use Committee
University of Pittsburgh, Suite 200, Hieber Bldg.

Instructions for Filling out this form

1. All teaching and research protocols utilizing living vertebrate animals must be tendered as New Submissions for complete evaluation by the Institutional Animal Care and Use Committee (IACUC). Applications must also be submitted when utilizing dead vertebrate animals or animal parts if the animal was both sacrificed and procured solely and specifically for the research or teaching purpose. All approved protocols must be re-submitted as a new protocol for review every three years.
2. All grants to be funded by the National Institutes of Health (NIH) must be reviewed *prior to* the release of funding to assure that all procedures on animals are included in an approved IACUC protocol. For this purpose, grant PIs must complete the "GRANT APPLICATION REVIEW FORM" and submit the form to the IACUC Office along with the grant application. The "GRANT APPLICATION REVIEW FORM" asks the PI to identify the IACUC protocols that correspond to the grant application animal work. A review of the grant submission and IACUC protocols will be performed to verify that all animal work has been described and approved by the IACUC. The Office of Research must certify that this review has been conducted before NIH will release grant funding. Therefore, all PIs receiving notice of a fundable score from NIH should contact the IACUC Office as soon as possible to initiate a review.
3. Download this Word and Excel based form at www.iacuc.pitt.edu and complete the entire application. Submit the completed application via email to iacuc@pitt.edu. Hardcopy applications will not be accepted. You will receive electronic confirmation of receipt within two days. Your application will be assigned to an appropriate subcommittee for review, and you may be asked to address reviewers' concerns. Following approval from all subcommittee members, you will receive an assurance letter. Should you wish to address the entire IACUC, please contact Dr. Ed Kennah at ekennah@pitt.edu or 383-2014. A minimum turn around time for protocol review is approximately 30 days. There are no expedited reviews since all protocols are required by regulation to go through the same review process.

4. All applications supported by an internal (Departmental) or corporate funding source must be accompanied by certification from the appropriate Dean, Department Head, or Division Chief that the protocol has been reviewed for scientific merit.
5. Postdoctoral fellows, graduate students, and undergraduate students submitting applications as principal investigators must also include sponsorship by an appropriate academic advisor. Please include the sponsor's name and contact information on the certification page of this application.
6. Protocols will not be approved for Principal Investigators who have not completed the required training programs. Contact Marilee Rose, Training Coordinator, at 578-3459 or mrose+@pitt.edu to arrange species-specific training. In addition, all personnel listed as participants in the study must complete the required training. Completion of Modules 1 and 3 is required training for all personnel listed as participants in the study. This can be accessed on the web at <http://www.health.pitt.edu/rpf/>.
7. Work involving rDNA must be approved by the Institutional Biosafety Committee for rDNA (Call 578-3799 for information and complete ~~Form 4~~ the Institutional Biosafety (Recombinant DNA) Application found at www.rcco.pitt.edu/rdna/).
8. If you place any of your animals in Pain and Distress Classification E, an explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on Attachment 1.
9. Work involving radiation must be approved by the Radiation Safety Office (Call 624-2728 for information and complete Attachment 2 of this form).
10. A Registration Workbook for Use of Biological and Chemical Agents must be completed for each IACUC application and approved by the Department of Environmental Health and Safety (see Attachment 3). IACUC protocols will not be reviewed until this Workbook is completed.
11. Investigators wishing to introduce any cell/tumor lines or bodily fluids of human or animal origin into animals in the DLAR, must first receive written permission from the Director of the DLAR or his designee before the first animal is injected. Attachment 4 must be completed.
12. Investigators requesting a dispensation from the nonhuman primate enrichment plan must complete Attachment 5. For information regarding enrichment techniques or any part of the enrichment plan, please contact Sarah Greene at serst28@pitt.edu , (412) 383-7892 or (724) 327-4607.

13. According to University guidelines, any persons receiving commercial funding must complete Module 4 on the Research Practice Fundamentals website - www.health.pitt.edu/rpf/. Investigators also need to complete Attachment 6 – Declaration of Conflict of Interest.

14. All Principal Investigators with approved protocols involving physical, chemical, and/or biological hazards must schedule a pre-study strategy meeting with the Division of Laboratory Animal Resources (DLAR) (648-8950) prior to ordering animals or initiating the project.

15. In an effort to limit the frequency of changes to IACUC submission forms, a single form will be used for each calendar year. Verify that you are using the current application form by checking the date at the top of this page. If changes are required during the year, the changes can be found under the "Additional Addendum" heading of the IACUC web site www.iacuc.pitt.edu/ Before submitting a protocol, check to ensure you are using the current form and that additional addendums (if required) are completed and attached.

16. Please type your response to the questions in the white space to the right of the question. (Do not type your response in the gray shaded areas.)

Consultation with a veterinarian from the DLAR prior to application submission is recommended. IACUC approval does not assure DLAR space availability.

A. Project Administration - Personnel:

1.	PI Name (First Name Last Name):	Adam Smith
2.	Co-PI 1 (First Name Last Name):	Bill Jones, Ph.D.
3.	Co-PI 2 (First Name Last Name):	
4.	Co-PI 3 (First Name Last Name):	
5.	PI Faculty Title:	Graduate Student Researcher
6.	PI Department:	Brain Function
7.	PI's Department Chair: (List Dean or Div. Chief if no Chair)	Dr. Alan Ericson / Smith Dr. Eugene Miles / Jones
8.	PI Mailing Address:	238 BST-75
9.	PI Office Phone:	412-647-0001
10.	PI Lab Phone:	412-647-2009
11.	PI Fax:	412-647-0008
12.	PI E-mail Address:	smith9@pitt.edu
13.	Emergency Contact (PI - 24 hr phone/pager):	412-551-8888 (PI's cell phone)
14.	Secondary Contact (CO – PI or Staff - 24 hr phone/pager):	412-889-0004 (Co-PI's cell phone)

B. Project Information:

15.	Title:	Role of Upper Cervical Inspiratory Neurons in Vestibulo-Respiratory Regulation			
16.	Source of Funding:	NIH			
17.	Total Project Period:	5/1/02-4/30/05			
18.	Grant Title and Number:	R01-DC03732, Vestibular Regulation of Respiratory Muscle Activity, to Bill Jones			
19.	Is the grant administered through the University of Pittsburgh?	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO			
20.	If No, what institution is administering the grant?	Not Applicable			
21.	Administering Institution's Protocol Approval # and Expiration Date:	Not Applicable			
22.	Administering Institution's USDA Registration #:	Not Applicable			
23.	Contact Person (name, phone, e-mail):	Not Applicable			
24.	Will any aspect of the animal experimentation be performed at another institution (including custom antibody production)?	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO			
If Yes, provide the following: (See www.aphis.usda.gov/ac/ Policy #10 for more information.)					
25.	Institution Name:	Not Applicable			
26.	Institution's IACUC Approval # and Expiration Date:	Not Applicable			
27.	USDA Registration #:	Not applicable			
28.	AAALAC Accreditation Status:	Not applicable			
29.	Contact Person (name, phone, e-mail):	Not Applicable			
<i>License and accreditation info available from the Office of Research at appropriate institution.</i>					
30.	Is this submission a major modification or a renewal of a currently active protocol?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
31.	If yes, please list the protocol #:				
32.	Please list the protocol's title:				

C. Research Staff (List all individuals handling live/dead animals or animal tissues, including PI)

33.	NAME	SOCIAL SEC # or PITT ID #	POSITION TITLE	*PROJECT ROLE	E-MAIL	PHONE/PAGE R #
	Adam Smith	666-66-6666	Graduate Student/PI	Experimental Procedures/ Data Analysis	smith9@pitt.edu	412-551-8888
	Bill Jones, Ph.D.	666-66-6666	Co-PI	Project oversight/Surge on	bjones@pitt.edu	412-647-0001
	Lucy Levy	666-66-6666	Research V	Animal Monitor	llevy@pitt.edu	412-647-2009
	Heather Arles	666-66-6666	Research III	Data Collection	Arles@pitt.edu	412-647-2009
	Katie Wiles	666-66-6666	Undergraduate Student	Data Collection	Wiles@pitt.edu	412-647-2009
	Brian Smithson	666-66-6666	Undergraduate Student	Data Collection	Smithson@pitt.edu	412-647-2009
	Mike Knoles	666-66-6666	Undergraduate Student	Data Collection	Knoles@pitt.edu	412-647-2009

*List the duties will this person perform relating to the animal studies.

D. Animal Usage:

Animals must be obtained from DLAR approved sources/vendors only. Quarantine may be required for certain animals based on their origin before they enter Institutional Animal Housing Space(s). Contact the DLAR (648-8950 or dlar@pitt.edu) for further information. Approximate weight range and age range of experimental subjects at time of arrival should be provided.

34. SPECIES	STRAIN	AGE	WGT.	SEX	Number projected for 3 Years*
CAT	---	Adult	> 3 KG	Either	20

*Only the number of individual animals of each species needs to be denoted here, not the number of individual animals of each strain.

E. Animal Housing And Use Sites:

Housing:

35. SPECIES	HOUSING LOCATION (Building, Room/Floor – if known)	HOUSING DURATION (Days)	MAXIMUM NUMBER ANIMALS HOUSED IN AN APPROVED FACILITY AT ONE TIME
CAT	CAF, BST-23, 9 th Floor	30 days	3

<p>36. Rodent Caging Requirements: (Complete only for Rodent protocols):</p> <p><input type="checkbox"/> Microisolator caging is required.</p> <p><input type="checkbox"/> Solid bottom caging is requested.</p> <p><input type="checkbox"/> Standard wire bottom caging is requested (see below.)</p> <p><input type="checkbox"/> Wire bottom caging is necessary, and I am requesting an exemption from the IACUC policy regarding use of this caging. (Provide justification below.)</p> <p><small>*IACUC policy states that animals exceeding 500 grams or maintained for > 9 months cannot be housed in wire-bottom caging unless a specific exemption is granted (See policy at www.iacuc.pitt.edu).</small></p> <p><i>If necessary, please provide scientific justification:</i></p>

F. Use Sites and Transportation Methods:

<p>37. Are animals transported from the holding room through approved animal facility space to another location/lab? [X] YES [] NO <small>(Approved animal facility space is defined as DLAR operated facilities as well as other animal housing spaces approved by the IACUC.)</small></p>
<p>38. If yes, indicate building(s) and room(s) of destination (If using hospital areas, see “Use of Experimental Animals in UPMC Hospital/Patient Areas policy at www.iacuc.pitt.edu/) 113 BST-75</p>
<p>39. Provide rationale for the need to remove animals from dedicated facilities and justify why such work cannot be performed in dedicated, approved animal facility space? These neurophysiological experiments require several racks of recording equipment and must be conducted in a shielded recording room. Also, a hydraulic tilt table is employed that weighs over a ton and is bolted to the foundation of the building to provide mechanical stability. It would thus be absolutely impossible to transfer this equipment to DLAR for experiments.</p>
<p>40. Will vertebrate animals be housed in these alternate locations for greater than 12 hours? Yes [] No [X] If yes, these sites must undergo additional evaluation and approval (contact the DLAR director at dlar@pitt.edu for further information).</p>
<p>41. Describe animal transportation route: Animals will be transported from the 9th to the 8th floor of DLAR in BST-23 via the service elevator. The 8th floor of DLAR has a connecting doorway to the BST-75, where our laboratory is located. A private elevator is available to transport the animals from the 8th floor DLAR to the hallway containing the PI’s lab. No contact with the general public occurs when this transport route is employed</p>
<p>42. Describe animal containment & transport mechanism: Cats will be placed in a conventional carrier that is draped with a blanket. The carrier is placed on a cart during transport. <small>(See Animal Transportation SOP at www.iacuc.pitt.edu/)</small></p>
<p>43. Indicate laboratory sites (if different than above) where hazardous tissues (e.g., nonhuman primate or tissues from studies involving the use of radiation, residual hazardous chemicals, or BSL-2 or greater containment) are to be taken: Not applicable Note that if the tissue(s) being transferred will be handled by investigators not listed on this protocol, you are required to inform that person(s) of any inherent dangers associated with the tissues. Your sign off on the certification Section Q verifies you have provided this information.</p>
<p>44. Describe tissue containment & transport mechanism: Not applicable</p>

G. Study Summary:

45. Briefly summarize (in less than three sentences) the aim(s) of the study, and why the study is important to human or animal health, the advancement of knowledge, or the good of society.

Summarize here: The overall aim of this experiment is to further our understanding of the role that the vestibular system plays in regulating breathing during movement and changes in posture. We will test the hypothesis that upper cervical inspiratory-related neurons relay vestibular signals to phrenic motoneurons. This study will improve our understanding of how the vestibular system participates in respiratory control, and is an important step in our continuing research in the field.

H. Animal Welfare Act Required Information:

46. Explain the rationale for animal use, including why non-animal models cannot be used:

Explain here: The proposed experiments will explore the neural pathways that mediate vestibular influences on respiration. This circuitry is poorly understood, and experiments in animals must be employed to address our aims. These neurophysiological experiments offer no alternative but to use an animal model to examine these physiological phenomena.

47. Justify appropriateness of the species selected and why a phylogenetically lower species could not be used?

Justify here: The neural circuitry engaged to regulate respiratory muscle activity is quite variable amongst mammals. Most mammals are not emetic, and lack respiratory control circuitry that is present in those species that can vomit. In addition, emetic species can employ their respiratory muscles for a host of behaviors including coughing and sneezing that are not present in non-emetic animals. The goals of these experiments require the use of an emetic animal species whose respiratory control circuitry is likely to be similar to that in the primate. This restriction excludes the use of most common laboratory animals, including mice, rats, and rabbits.

The cat has been the typical model animal for studies of the neural control of respiration. Cats are emetic, and appear to employ their respiratory muscles for similar behaviors as primates, including humans. Thus, a considerable database of information is available regarding the control of respiration in this species. Many physiological studies regarding vestibular control of respiration have been performed in the cat. Background information that leads to the present experiments is unavailable for all species other than the cat.

48. Number of experimental/control groups*: 1

49. Number of animals per group*: 20

*Number of Experimental/Control Groups X Number of Animals per group should equal the total number of animals delineated in the responses to Question 34.

**50. How was sample size determined? (Answer by placing an X in the appropriate bracket(s))
(The number of animals requested should be the minimum required for statistically valid results.)***

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Numbers and Species of Animals and <ftp://ftp.grants.nih.gov/IACUC/GuideBook.pdf> Fundamental Issues, Criteria)

Pilot study. No report of a similar study exists. The proposed study will utilize a small number of animals (typically less than 10) to obtain preliminary data to justify the

performance of a larger study.

Previous experience by this PI (if checked, answer question 51)

Studies cited in the literature (If checked, answer question 51)

51. If previous experience and/or literature review was used to determine sample size, a power analysis should be described if possible, citing type of statistical analysis used. If the experimental approach is not amenable to a power analysis, a thorough justification of the number of animals required must be provided.

Describe and Justify here: The sample size for this experiment is not based upon animal numbers, but on the number of premotor upper cervical neurons whose responses to vestibular stimulation can be characterized. Based on previous studies, we expect to identify approximately 2-5 neurons whose axons can be microstimulated within the phrenic motor nucleus in a particular experiment.

Most neurophysiological studies of the type proposed here require sampling of activity of at least 50 neurons. Thus, if 2-5 units are characterized per animal, a total of 20 animals will be required to complete this study. This yield of upper cervical inspiratory neurons with projections to the phrenic motor pool is predicted from the following references:

[1] Aoki, M., Mori, S., Kawahara, K., Watanabe, H. and Ebata, N., Generation of spontaneous respiratory rhythm in high spinal cats, *Brain Res*, 202 (1980) 51-63.

[2] Gang, S., Sato, Y., Kohama, I. and Aoki, M., Afferent projections to the Botzinger complex from the upper cervical cord and other respiratory related structures in the brainstem in cats: retrograde WGA-HRP tracing, *J Auton Nerv Syst*, 56 (1995) 1-7.

[3] Nakazono, Y. and Aoki, M., Excitatory connections between upper cervical inspiratory neurons and phrenic motoneurons in cats, *J Appl Physiol*, 77 (1994) 679-83.

[4] Satomi, H., Takahashi, K., Aoki, M., Kasaba, T., Kurosawa, Y. and Otsuka, K., Localization of the spinal accessory motoneurons in the cervical cord in connection with the phrenic nucleus: an HRP study in cats, *Brain Res*, 344 (1985) 227-30.

A literature search that further justifies the usage of 20 animals in this experiment is provided below in item 95.

I. Drug Administration

Anesthesia, Analgesia, Tranquilizing, and Paralytic Drugs (Specify dosage in mg/kg)

52. SPECIES	DRUG	DOSE	ROUTE	ADMIN. FREQUENCY
Cat	Isoflurane	5% Induction; 1-3% maintenance	Inhaled	Continuously during surgery, but discontinued following decerebration
Cat	Gallamine triethiodide	5 mg/kg initial dose; 2.5 mg/kg maintenance	IV	Every hour

For drugs listed above, please supply the duration of treatment and method of monitoring efficacy:

53. DRUG	Duration of Treatment	Method of Monitoring Efficacy
Isoflurane	Continuously during surgery	Blood pressure, respiration rate, absence of withdrawal reflexes
Gallamine triethiodide	After decerebration until end of experiment	Absence of movement or attempt to breathe against respirator

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> -Minimization of Pain & Distress)

54. Are Paralytic Drugs Used: Yes No
If Yes, paralytic drug used: [Gallamine triethiodide]

Describe Health Monitoring Procedures: This experiment employs the use of the decerebrate preparation, which is clinically dead (brain structures rostral to the superior colliculus are either removed or rendered dysfunctional). The decerebrate preparation is not anesthetized because it cannot experience pain or discomfort. Paralysis will only be performed after the decerebration is complete and deemed to be successful. Thus, in these experiments we need not be concerned about whether animals recover from anesthesia while under paralysis.

While animals are paralyzed, they will be artificially ventilated at an approximate rate of 20/minute. End tidal CO₂ will be monitored and maintained near 4% to assure that ventilation is adequate.

- a. Justification for use of the paralytic agent in this study.
- b. Provide information and/or data that demonstrate the proposed anesthetic regimen is adequate without the paralytic agent. This can be based on a) literature citations specifically using the same regimen, b) documented experience of the PI with this anesthetic regimen without paralytic inclusion or c) the previous documented performance of a "sham" procedure utilizing the regimen without paralytic agent use.
- c. Provide a plan to titrate the amount of anesthetic to establish a verifiably adequate plane of anesthesia in each animal prior to inducing paralysis.
- d. Describe the proposed anesthetic monitoring procedures in paralyzed animals (e.g. electroencephalogram, electrocardiogram, blood pressure, etc.) Please note if heart rate or blood pressure is monitored, elevations or depressions of 15-20% from baseline prior to adding paralytics may be indicative of need for alteration of anesthetic levels.

a. The proposed neurophysiological experiments involve stimulation within the spinal

cord to identify upper cervical neurons whose axons project to the vicinity of respiratory motoneurons. Such stimulation would induce movement of the animals, and thus neurons that are being recorded from would be lost. It is only possible to conduct the study we are proposing if animals are paralyzed.

- b. We are employing a decerebrate preparation that is clinically dead and is not anesthetized. Thus, we need not be concerned about anesthetic levels during paralysis. The Co-PI has substantial experience utilizing this anesthetic regime.
- c. As indicated above, we are employing a decerebrate preparation that is clinically dead and is not anesthetized. Thus, we need not be concerned about anesthetic levels during paralysis.
- d. Although the decerebrate animals are clinically dead, we monitor their condition carefully. Blood pressure is monitored and supported if necessary by infusing a solution of phenylephrine, an alpha adrenergic agonist. In addition, end tidal CO₂ is monitored and maintained near 4% by adjusting the ventilation volume.

55. Drugs Administered for Therapeutic Purposes (excluding anesthetics, analgesics, and tranquilizers)

Will pharmaceutical agents be introduced to the living and/or dead animals? YES [] NO
If Yes,

SPECIES	AGENTS	DOSE	ROUTE	EFFECT
Cat	Phenylephrine	Infusion of up to 1 mg/kg/hr	IV	Increase blood pressure if animal becomes hypotensive
Cat	Atropine sulfate	0.15 mg/kg	IM every 6 hours	Employed to reduce airway secretions.

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable: Phenylephrine is an adrenergic agonist, and produces considerable vasoconstriction and increases in blood pressure. An overdose can produce hypertension. We will guard against this by infusing the drug slowly and monitoring blood pressure continuously during delivery.

56. Are any *non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) regulated species (vertebrate animals excluding rats, mice and birds)? YES [] NO

*(Please note: As per specific Animal Welfare Act directive, "Non-pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. **Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in regulated animals.**")*

(See policy at www.pitt.edu/policies.htm)

***Non-pharmaceutical agent** – An agent that was not specifically prepared for injection into animal or human in its current form.

If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.

Describe: USDA policy 3 explicitly states that pharmaceutical grade agents must be used in regulated species if available. However, the paralytic drug that we employ in these studies, gallamine triethiodide, is no longer available in pharmaceutical grade. We are thus petitioning

the IACUC to allow us to use a non-pharmaceutical grade version of the chemical compound obtained from Sigma.

Gallamine triethiodide is the paralytic drug of choice for us, because of its extremely long time course. We have considered the pharmaceutical grade paralytics remaining on the market (e.g., Anectine), and all have a much shorter half-life. It is imperative for the sake of these experiments that animals do not recover from paralysis, and thus a long-lasting drug is critical.

Paralysis is only employed in these experiments after animals have been rendered decerebrate, and thus from a legal sense are “clinically dead.” Once portions of the brain rostral to the brainstem are removed, animals can no longer sense pain or discomfort.

Gallamine triethiodide will be dissolved in sterile saline on the day of the experiment, and any unused solution will be discarded at the end of the nonrecovery procedure. Although the gallamine triethiodide obtained from Sigma has no designated “expiration date,” our laboratory practice will be never to maintain a container of the chemical compound for more than 12 months.

57. Are non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) non-regulated species (rats, mice or birds) for the purpose of veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics)? [] YES [X] NO

(Please note: As per University of Pittsburgh IACUC approved policy, all compounds used in rats, mice or birds for veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics) must be pharmaceutical grade (when available) unless scientifically justified. Cost savings alone are not considered by the IACUC an adequate justification for using non-pharmaceutical grade drugs for veterinary medical care or to relieve pain and distress in these species.)

(See policy at www.pitt.edu/policies.htm)

If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.

Describe: NOT APPLICABLE—WE ARE USING AN AWA-REGULATED SPECIES

J. Euthanasia

58. Will animals be euthanized?	[X] YES [] NO
59. If NO, what is final disposition of animals?	
60. If YES, provide drug and dose or method used.	IV injection of 120 mg/kg sodium pentobarbital
See http://www.avma.org/resources/euthanasia.pdf for approved euthanasia methods (Adobe Acrobat software required)	

K. Animal Exposures

Radioactive Agents (Prior to any study involving the administration of radioisotopes to animals, the Radiation Safety Office must review and approve the use.)

61. Will radioactive substances be administered in live animals? YES NO
If Yes, complete Attachment 2 and the table below.

SPECIES	ISOTOPE	ACTIVITY

62. Will X-Ray equipment, accelerators, CT scanners, irradiators, or external beam radiation be used? YES NO
If Yes, complete the table below. (It is not necessary to complete Attachment 2 for a Yes response to this question.)

EQUIPMENT	LOCATION OF USE	DOSE*

*Dose not applicable to diagnostic imaging studies

Biological Agents (complete Attachment 3)

63. Will Biological Agents be used in living and/or dead animals? YES NO
If Yes,

SPECIES	BIOLOGICALS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

64. Chemical Agents (Do not include drugs used for therapeutic purposes) ?

Will chemical agents be introduced to the living and/or dead animals? YES NO
If Yes,

SPECIES	CHEMICALS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

65. Cells/Tumor Lines/Bodily Fluids (complete Attachment 4)

Will cells, tumor lines, or bodily fluids be introduced to the live and/or dead animals? YES
 NO
If Yes,

SPECIES	AGENTS	DOSE	ROUTE	EFFECT

L. Description of Experimental Design and Animal Procedures:

66. Describe the specific experimental manipulations and treatments of the animals in terms that are intelligible to non-specialists. This description should allow the IACUC reviewer to understand exactly what will be done to all the animals from entry into the study to the endpoint of the study. It would be helpful to provide a flow chart or table illustrating your experimental design and include animal numbers to be used. The experimental endpoint that is being measured must be indicated for each procedure. (Please do not insert the Methods Section from the grant application. The IACUC is only required to review procedures that are done to animals.)

Describe Experimental Design, Animal Procedures, and Surgical Procedures here: Animals will be transported from the CAF to the PI's laboratory in the BST-75 Building on the day of the nonrecovery experiment. Animals will be placed in an anesthetic chamber, and anesthetized using Isoflurane (5%) vaporized in oxygen and nitrous oxide. After induction of anesthesia, the anesthesia will be administered through a face mask (which seals tightly around the animal's nose and mouth to prevent escape of gases); the level of anesthetic will be adjusted to maintain areflexia and a stable respiration rate. A vacuum scavenging system is employed to remove any gases that may bleed off from the relief valve of the anesthesia machine. It is our experience that a 1.5-2% concentration of isoflurane is required to maintain deep anesthesia when administered through a face mask. Subsequently, a blood pressure transducer will be inserted into a femoral artery, so that arterial blood pressure can be monitored as an indicator of anesthetic depth; the level of isoflurane will be adjusted to maintain blood pressure near 100 mm Hg. We will also continue to monitor respiration rate and the absence of withdrawal reflexes as an indicator that anesthetic depth is appropriate.

Subsequently, both femoral veins will be cannulated to allow for i.v. administration of fluids and drugs, and a tracheostomy will be performed. After this point, we will begin administering anesthesia through the tracheal cannula. Both carotid arteries will be ligated, a craniotomy will be performed, and the posterior occipital cortex will be aspirated to expose the rostral brainstem. A decerebration will be performed by cutting the brainstem at the midcollicular level. At least 1 cm of brain tissue rostral to the decerebration will be aspirated to assure that the decerebration is complete. Subsequently, anesthesia will be removed. Decerebrate animals are known to be in a persistent vegetative state, and do not experience pain or discomfort. Thus, it is humane to remove anesthesia from a decerebrate preparation. Furthermore, the Co-PI has employed the decerebrate cat preparation for over 20 years, and has performed over 500 experiments using this preparation.

The most caudal portion of the brainstem will be exposed, and a laminectomy from the base of the brainstem down to approximately the T1 level will be performed to expose the dorsal surface of the cervical spinal cord to provide access for neurophysiological recordings from upper cervical neurons, and to place a stimulating microelectrode in the phrenic motor pool at the C5-C6 spinal level. In addition, the phrenic, hypoglossal, and abdominal nerves will be dissected so that respiratory-related activity can be monitored. In 10 animals, stimulating electrodes will also be implanted bilaterally into the vestibular labyrinth near the 8th cranial nerve and secured using dental cement.

Following the completion of all surgical procedures, animals will be paralyzed using an i.v. injection of 5 mg/kg of gallamine triethiodide; paralyzed animals will be artificially respired using a positive-pressure ventilator (approximately 20 cycles/minute). Paralysis will NEVER be performed prior to the decerebration, so that withdrawal reflexes can be monitored as an indicator of depth of anesthesia. End-tidal CO₂ will be monitored following paralysis and maintained near 4%.

The goal of this experiment is to identify upper cervical premotor neurons that project to the phrenic motor pool, and control the activity of diaphragm motoneurons. Microstimulation within the phrenic motor nucleus will be employed to identify upper cervical neurons that participate in regulating respiratory activity. For 10 animals, after a premotor respiratory neuron is isolated, we will record its responses to whole-body tilt delivered using our hydraulic tilt-table. This device can produce rotations in the pitch and roll planes at frequencies up to 1 Hz and at amplitudes up to 20°. By recording the neuron's responses to these rotations, we can determine whether the vestibular system affects its activity and if so which vestibular endorgans contribute inputs to the cell. For the other 10 animals, the threshold of response to electrical vestibular stimulation will be determined during the surgical procedures. After a premotor respiratory neuron is located, the vestibular nerve will be stimulated according to its predetermined threshold, and the neuron's responses to stimulation will be recorded in order to determine if these cells respond to vestibular signals.

Throughout the experiment, fluids will be administered i.v. to replace loss. Hourly injections of gallamine triethiodide (2.5 mg/kg) will be performed to maintain paralysis. Rectal temperature will be monitored and maintained near 38°C using a heating pad and infrared lamp. If blood pressure drops below 100 mm Hg, the alpha-agonist phenylephrine will be infused, as necessary, to produce vasoconstriction and thus increase blood pressure.

At the end of the recording session, animals will be euthanized using an overdose (120 mg/kg i.v.) of sodium pentobarbital. We will monitor blood pressure and end-tidal CO₂ after injection of sodium pentobarbital to assure that the animal has been killed. In most experiments, the brainstem will be removed for reconstruction of recording sites.

67. Describe Experimental Endpoints Here: (At what point is the experiment completed on animals?) These nonrecovery experiments will continue as long as 1) central respiratory output is maintained, as indicated by the presence of respiratory-related discharges on phrenic, hypoglossal, and abdominal nerve recordings and 2) blood pressure can be maintained at 100 mm Hg by infusion of no more than 1 mg/kg/hr of phenylephrine. It is anticipated that a recording session will continue for about 12 hours, but may last as long as 24 hours.

For each <i>Non-surgical Procedure</i> for each species: Specifically address the following:
68. Will blood sampling be conducted? [] Yes [X] No
69. If Yes, provide rationale, method, site, volume, & frequency:
70. Will food scheduling or restriction (other than standard pre-operative fasting) be conducted? [] Yes [X] No
71. Provide rationale, method, frequency, & duration:
72. Will water scheduling or restriction (other than standard pre-operative fasting) be conducted? [] Yes [X] No
73. If Yes, provide rationale, method, frequency, & duration:
74. Will restraint methods be utilized? [] Yes [X] No
75. If Yes, provide rationale, method, frequency, & duration*:
*Note: Provide a detailed description of non-human primate handling methods (other than chemical immobilization) and justify the use of alternative methods and provide assurances of personnel safety.
76. Will stress paradigms be utilized? [] Yes [X] No
77. If Yes, provide rationale, type, frequency, & duration:

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Methodology)

For Each <i>Surgical Procedure</i> for each species, specifically address the following (required): (The Surgical Guidelines are available at www.iacuc.pitt.edu under "Policies.")
78. Will non-survival surgery be performed? Yes [X] No []
79. Will survival surgery be performed? Yes [] No [X]
80. Will multiple survival surgeries be performed on one animal? Yes [] No [X] (See www.aphis.usda.gov/ac/ -Policy #14.)
81. If yes to question 80, please justify: <i>Enter justification here:</i>
82. Surgical Site (Building, Room Number): 113 BST-75
83. Names of Surgeon(s): Bill Jones, Ph.D., and Adam Smith
84. Has the surgeon(s) performed this procedure on the species requested? Yes [] No [X]
85. If no to question 84, please provide training plan: Bill Jones has significant experience performing these surgical procedures in the cat. The PI, Adam Smith, is a graduate student in his laboratory with very limited surgical experience, and will be trained in the above described surgical procedures by Bill Jones. Adam will only perform these procedures, including decerebration, under the close supervision of Bill Jones. Dr. Jones will be present during every surgical procedure.
86. Postoperative Plan (Methods to assess/alleviate pain/distress, Recovery Criteria, Monitoring Criteria - See http://grants.nih.gov/grants/olaw/GuideBook.pdf):
<i>Enter plans for monitoring animals as well as alleviating pain and distress here:</i> Not applicable; these are non-recovery experiments.

M. Anticipated Effects on Experimental Animals

87. Changes to Health and Well-Being

Please describe all signs of morbidity (e.g., decreased food and/or water consumption, weakness, infection neoplasia, neurological effects, etc.) expected to occur in the experimental subjects as a result of the procedures performed in this protocol (If mortality is expected please note here and see [http://www.iacuc.pitt.edu/sop/Pain %20Indicators.htm](http://www.iacuc.pitt.edu/sop/Pain_%20Indicators.htm).) Although the Co-PI has considerable experience using the decerebrate preparation (hundreds of animals), experience shows that there is ~10% mortality associated with the decerebration procedure. Most cats are amenable to decerebration because they have minimum blood flow through the Circle of Willis; after ligating the carotid arteries it is typically possible to transect the brainstem at a mid-collicular level without producing significant bleeding. However, in some cats the Circle of Willis remains patent and bleeding occurs following the decerebration. This is the single largest problem anticipated in these experiments.

88. Describe the expected frequency of the complications noted in question 87 and how you will deal with these complications:

Describe here: When bleeding occurs at the decerebration site, we will increase the anesthesia to produce a temporary hypotensive state. When bleeding is minimized by reducing blood pressure, we will attempt to isolate the bleeding arteries and cauterize them. This can usually be accomplished, but in some cases (~10%) animals may die during the decerebration.

Please complete the following two questions for all procedures.

89. Methods and Frequency of Monitoring Health/Well-Being Changes

Describe in detail the species specific procedures that the investigator will use to monitor the experimental animals' health and well-being, monitor pain and distress, alleviate pain and distress, and the frequency with which these procedures will be performed: Arterial blood pressure is recorded continuously during the study and displayed on a panel so that it can be read easily throughout the laboratory. Our blood pressure recording unit has audible alarms such that we are alerted immediately when blood pressure is altered. Furthermore, end tidal CO₂ is monitored continuously and displayed such that it can be read easily anywhere in the laboratory. Audible alarms on the CO₂ unit will also alert us when this parameter changes, so that we can adjust ventilation volume appropriately. As mentioned above, decerebrate animals are known to be in a persistent vegetative state, and do not experience pain or discomfort. Thus, following decerebration, no procedure will be necessary to monitor or alleviate pain or distress.

During surgery (prior to decerebration), heart rate, respiration rate, blood pressure, and rectal temperature will be recorded at least every 15 minutes and reported to DLAR using the standard anesthetic monitoring form.

90. Criteria for Premature Removal of Animals from Study (This section applies to acute and chronic studies)

Describe in detail the criteria utilized to determine that an experimental animal must be removed from a study: These nonrecovery experiments will continue as long as 1) central respiratory output is maintained as indicated by the presence of respiratory-related discharges on nerve recordings and 2) blood pressure can be maintained at 100 mm Hg by infusion of no more than 1 mg/kg/hr of phenylephrine. When the animal's condition deteriorates such that blood pressure drops or central respiratory activity disappears, it will immediately be euthanized.

N. Pain or Distress Classification

Please note that this section applies to USDA and Public Health Service (NIH) regulated species. This includes all species of animals and is a change from an earlier application.

Pain or Distress Classification

91.	SPECIES (Common Name)	USDA CLASSIFICATION* % of Animals Classified			
		B	C	D	E
	Cat	%	%	100%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%

* **USDA Classifications** (see www.aphis.usda.gov/ac/ -Policy #11 for more information)

Classification B: Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery, but not yet used for such purposes.

Classification C: Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, no distress, or no use of pain-relieving drugs. Euthanasia must precede any invasive procedure (i.e. tissue harvesting) to be in Classification C.

Classification D: Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used. Acute or terminal surgery is considered a painful procedure, which is alleviated by anesthesia.

Classification E: Animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests and/or animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted resulting in permanent physiological impairment that may lead to chronic pain or distress.

NOTE REGARDING CLASSIFICATION E: An explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on **Attachment 1**. This information is required to be reported to the USDA, will be available from the USDA under the Freedom of Information Act, and may be publicly available through the Internet via USDA's website.

O. Consideration of Alternatives To Painful/Distressful Procedures –

(Complete only if you are using vertebrate species other than purpose-bred birds, mice of the genus *Mus*, and rats of the genus *Rattus*)

This section applies to all USDA regulated species. Reference Librarian, Alice Kuller is available for consultation in performing literature searches (648-1971).

If any procedures fall into USDA's Classification D or E, causing more than momentary or slight pain or distress to the animals, USDA Policy 12 requires that you must address **all three** of the following issues:

- 1) You have refined potential pain-producing methods as much as possible to minimize distress.
- 2) You have reduced animal use as much as possible without jeopardizing statistical validity.
- 3) You have considered replacing potential pain-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

As part of the response, you must provide written narratives that will convince the IACUC reviewers you have made a good faith effort to substantively address **each** of these three issues. You must also provide **two sources** of information to show that you have addressed **each** of the three issues. At least one of these sources of information should be a search of a scientific database. For each search executed, you must specify the **database** considered, the **date** of the search, **period** covered by the database searched, and the **keywords** used. If you elect to search two databases to satisfy the consideration of alternatives, please note that both Medline and PUBMED are search engines that consider the same database, the National Library of Medicine, and thus employing both of these search engines is considered to be a single database search. **Note: the investigator should retain all searches, as they are reviewable by federal agencies.**

An alternate method of consideration for alternatives is consulting with an expert (e.g., DLAR veterinarian, another PI with extensive experience with the issue in question) or attending conferences or colloquia that provide relevant and up-to-date information directly related to the issue. When sources other than database searches are included, sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge on the availability of alternatives in the specific field of study.

For more information, see www.aphis.usda.gov/ac/ -Policy #12.

Instructions:

For each section below, provide at least 2 database searches, or 1 database search + an expert consultation, then provide a written narrative.

92. Refinement - Provide evidence that you have refined potential pain or distress-producing procedures as much as possible to minimize distress.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
1/20/04	Medline	1966 through January 2004	cat, anesthesia,

			decerebrate
1/20/04	Alt Web	Entire Database	cat, isoflurane, decerebrate

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation
Not applicable			

Written narrative based on this information:

Medline: anesthesia + cat = 705 hits; decerebrate + cat = 686 hits; isoflurane + cat = 31 hits.
 AltWeb: isoflurane + cat = 31 hits; decerebrate = 9 hits

These searches revealed that the procedures we have chosen to reduce pain and distress in this study are very effective. Isoflurane has been well-established for use in the cat, and has proven very effective. Also, a great amount of literature demonstrates that decerebrate animals do not suffer pain or distress, thus eliminating the need for anesthesia. This procedure cannot be refined further.

93. Reduction - Provide evidence that you have reduced animal use as much as possible without jeopardizing statistical validity.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
1/20/04	Medline	1966 through January 2004	cat, respiration, vestibular
1/20/04	Agricola	Entire Database	respiration, regulation

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation
Not applicable			

Written narrative based on this information:

Medline: respiration + cat + vestibular = 7 hits
 Agricola: respiration + regulation = 25 hits

Relevant references:

- [1] Aoki, M., Mori, S., Kawahara, K., Watanabe, H. and Ebata, N., Generation of spontaneous respiratory rhythm in high spinal cats, *Brain Res*, 202 (1980) 51-63.
- [2] Gang, S., Sato, Y., Kohama, I. and Aoki, M., Afferent projections to the Botzinger complex from the upper cervical cord and other respiratory related structures in the brainstem in cats: retrograde WGA-HRP tracing, *J Auton Nerv Syst*, 56 (1995) 1-7.
- [3] Nakazono, Y. and Aoki, M., Excitatory connections between upper cervical inspiratory neurons and phrenic motoneurons in cats, *J Appl Physiol*, 77 (1994) 679-83.
- [4] Satomi, H., Takahashi, K., Aoki, M., Kasaba, T., Kurosawa, Y. and Otsuka, K., Localization of the spinal accessory motoneurons in the cervical cord in connection with the phrenic nucleus: an HRP study in cats, *Brain Res*, 344 (1985) 227-30.

The literature search revealed that responses of approximately 50 brainstem units are typically analyzed for a study of the type proposed in this application. In addition, the references listed above suggest that approximately 20 animals will be required to isolate and study vestibular inputs to 50 upper cervical spinal neurons with projections to the phrenic motor pool. Thus, the literature search showed that animal numbers cannot be reduced without jeopardizing the scientific validity of the study.

94. Replacement - Provide evidence that you have considered replacing potential pain or distress-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
1/20/04	Medline	1966 through January 2004	cat, respiration, vestibular
1/20/04	Agricola	Entire Database	respiration, regulation

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation
Not Applicable			

Written narrative based on this information:

Medline: respiration + cat + vestibular = 7 hits
 Agricola: respiration + regulation = 25 hits

The literature search revealed that limited information is available regarding the neural circuitry that mediates vestibular influences on respiration. Currently, there are no data regarding potential vestibular influences on upper cervical inspiratory neurons, which are the neurons of interest in this study. Thus, methods such as computer modeling cannot achieve the goals of this study, due to the lack of sufficient background data.

P. Exemption from Environmental Enrichment For Nonhuman Primates or Exercise Program for Dogs

<p>95. For nonhuman primates, are you seeking an exemption from the institution’s plan for environmental enrichment? (see Plan to Promote the Psychological Well-Being of Nonhuman Primates at www.iacuc.pitt.edu/policies.htm) <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, complete Attachment5</p>
<p>96. For dogs, are you seeking an exemption from the institution’s plan for exercise (see www.iacuc.pitt.edu/policies.htm)? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, justify below:</p>
<p><i>Type Justification Here</i></p>

Q. Principal Investigator Certifications

- I certify that I have determined that the research proposed herein is not unnecessarily duplicative.
- I certify that I will notify the IACUC before initiating any significant changes in this protocol.

- I certify that I will notify the IACUC regarding any unexpected results that impact animals. Any unanticipated pain, distress, morbidity or mortality will be reported to the attending veterinarian.
- I understand that if I cannot be contacted in the event that animals in this project show evidence of distress, illness or pain, emergency care, including euthanasia if necessary, will be administered at the discretion of the veterinary medical staff.
- For all USDA Classification D and E proposals (see Section N): I certify that I have reviewed the pertinent scientific literature and the sources and/or databases as noted in Section O and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
- For any animal body fluids or tissues, derived from the studies described in this application and transferred to persons not listed in Section C, I certify I will notified the recipient of all hazards associated with these materials and instructed them to register with the EHS Department (624-9505)
- I certify that I will share a copy of the approved protocol with all personnel identified in Section C and they will read and understand all elements described for the study.
- I certify that all surgeons involved in this project have read the Surgical Guidelines. (The Surgical Guidelines are available at www.iacuc.pitt.edu under "Policies."

R. Inclusions*: (Please respond by placing an X in the appropriate bracket):

97. This protocol involves animals in Pain or Distress Classification E: [] YES [X] NO
If yes, has Attachment 1 been completed? [] YES [] NO
98. This protocol involves radiation usage: [] YES [X] NO
If yes, is this protocol registered with the Radiation Safety Office (Attachment 2)?
[] YES [] NO
99. This protocol involves hazardous chemicals or biologicals: [X] YES [] NO
If yes, is this protocol registered with the Health and Safety Office (Attachment 3)
[X] YES [] NO
100. This protocol introduces cells/tumor lines or bodily fluids into animals: [] YES [X] NO
If yes, is this protocol registered with the DLAR (Attachment 4)? [] YES [] NO
101. This protocol seeks an exemption from the institution's environmental enrichment plan for nonhuman primates: [] YES [X] NO
If yes, has a dispensation from the University of Pittsburgh's Nonhuman Primate Enrichment Plan (Attachment 5) has been requested? [] YES [] NO
102. This protocol involves private or commercial funding: [] YES [X] NO
If yes, has the Conflict of Interest Form has been completed (Attachment 6)?
[] YES [] NO
103. This protocol involves rDNA: [] YES [X] NO
If yes, has registration been submitted to the IBC? [] YES [] NO

If the rDNA is already registered, has the IBC been notified to add this protocol to your registration? YES NO

Note – Please delete all Attachments not valid to your protocol before returning this completed form to the IACUC office.

Principal Investigator Certification: (First Name Last Name):

Adam Smith

Date Submitted:

January 22, 2004

If Applicable, Post-Doctorate and Graduate Student Sponsor (Name, phone #, e-mail address): Bill Jones, Ph.D. Phone # 412-647-9614. email: bJones@pitt.edu

As a sponsor, I agree to take responsibility for all experimental studies performed pertaining to this protocol and agree to comply with all federal regulations applicable to this research YES NO