[redacted]
05006-18►
Official Date of Approval►

ANIMAL COMPONENT OF RESEARCH PROTOCOL(ACORP)

Main Body
VERSION 4 v 2 6-17-2015

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

A. ACORP Status.

1. Full Name of Principal Investigator(s)► [redacted] **Ph.D.**
2. VA Station Name (City) and 3-Digit Station Number► **VA Greater Los Angeles 691**
3. **Protocol Title► GABAergic Switches Control Wakefulness, NREM sleep and REM sleep**
4. Animal Species covered by this ACORP► Cat
5. Funding Source(s). Check each source that applies:

   ►( ) Department of Veterans Affairs.
   ►(X) US Public Health Service (e.g. NIH).
   ►( ) Private or Charitable Foundation -- Identify the Foundation:
   ►( ) University Intramural Funds – Identify the University and Funding Component:
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review: ► **NO** (X) -- **GO TO Item #7**
   Else, identify the project:
   (1) Title of project►
   (2) If approved by the R&D Committee, give the date of approval►

NOTE TO REVIEWERS: Part 6b differs from version 3 ACORP. This is basically a progress report. Reviewers should consider if this project has been inactive and flag that for discussion by committee.

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:
   (1) Identify the studies described in the previously approved ACORP that have already been completed

► Study 1: Electrophysiological identification of the cellular mechanisms of GABAergic processes in the control of activity of neurons in the nucleus pontis oralis

The present study was designed to explore the cellular mechanisms of GABA actions on neurons that generate REM sleep (REM-generator neurons) in the nucleus pontis oralis (NPO), and provide the electrophysiological evidence that the effects of GABA are due to a direct inhibitory action on NPO REM-generator neurons. Accordingly, the effects of the juxtacellular application of GABA and bicuculline, a GABA-A antagonist, on the activity of REM-generator neurons which were recorded intracellularly in the NPO were examined. The juxtacellular application of GABA hyperpolarized the membrane potential of NPO neurons, and significantly decreased the amplitude of spontaneous EPSPs and the frequency of discharge of these cells; in contrast, the juxtacellular microejection of bicuculline depolarized NPO neurons and significantly increased the amplitude of spontaneous EPSPs and the frequency of their discharge. The present results demonstrate, at a single cellular level of analysis, that inhibitory GABAergic inputs are capable of

controlling the activity and discharge frequency of REM-generator neurons in the NPO, and indicate that these NPO neurons are under tonic GABAergic inhibitory control during wakefulness. Therefore, we believe that the pontine GABAergic mechanism functions in such a way that wakefulness is induced and maintained due to the activation of GABAergic process, which results in the suppression of discharge of REM-Generator neurons in the NPO.

►Study 2: Anatomical evidence of direct hypocretinergic (orexinergic) control of GABAergic neurons in the nucleus pontis oralis

A large number of studies have implicated the hypocretin (orexin) system as a critical regulator of sleep/wake states as well as feeding behavior and reward processes. On the other hand, a plethora of data demonstrates that GABAergic inhibitory processes in the nucleus pontis oralis (NPO) play an important role in the generation and maintenance of wakefulness as well as REM sleep. Accordingly, the present study was undertaken to investigate whether there is any direct interaction of the hypocretinergic neurons and GABAergic neurons that comprise the pontine GABAergic system which functions to promote wakefulness and suppress REM sleep in the NPO. The animals were deeply anesthetized and perfused for immunohistochemical studies. GABA and hypocretin receptor-1 (Hcrtr1) or receptor-2 (Hcrtr2) imunore activities in the brainstem sections containing the NPO were examined following the procedure of double fluorescence immunohistochemistry. Under fluorescence microscopy, neurons exhibiting both GABA and Hcrtr1 or GABA and Hcrtr2 immunoreactivities were observed in the NPO, indicating that GABAergic neurons in the NPO receive direct hypocretinergic inputs via both receptors. However, the number of Hcrtr1 immunoreactivities that expressed on NPO GABAergic neurons was greater than Hcrtr2. The present results demonstrate that GABAergic neurons in the NPO is under direct influence of the hypocretinergic inputs and such a direct control is mediated by both hypocretin receptor-1 and receptor-2. Therefore, we suggest that the hypocretinergic control of wakefulness and REM sleep is partly mediated via direct activation of pontine GABAergic neurons by the hypocretins.

(2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly

► **Four**

(3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other items below.

► **We have modified the drug list for our study to be more up to date on modern standard practice and use drugs that are less harmful to the animal. We have substituted carprofen for another Post-Analgesic NSAID, meloxicam. We have also substituted ketamine and xylazine (tranquilizer) with a more modern method referred to as DKT, combining equal parts dexmedetomidine, ketamine, and butorphanol. This method allows atipamezole to be used as a reversing agent to dexmedetomidine once the surgery is complete.**

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).
(NOTE TO REVIEWERS: This is section would only be filled out under specific circumstances. This section should normally be left blank)
(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):
   - ►(X) Research
   - ►( ) Teaching or Training
   - ►( ) Testing
   - ►( ) Breeding and colony management only; not for any specific research project
   - ►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
   - ►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of veterans, the general population and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

(NOTE TO REVIEWERS: Please check that benefits to the veteran population is specifically addressed in this section)

► Trouble falling and staying asleep are common in mood and anxiety disorders such as depression and PTSD. Among active duty military, insomnia is one of the most frequent reasons for mental health referrals and is among the most frequent complaints of recently deployed Veterans. One example of this is E.R., a soldier who served two tours of duty in Afghanistan. Upon returning home E.R. struggles to maintain a "normal" sleep schedule. He has difficulty falling asleep at night, rarely sleeps for more than a few hours at a time, and reports distressing combat-related nightmares. The insomnia and nightmares make it difficult for him to focus on schoolwork and he relies on energy drinks, several per day, to stay awake and alert even though he has been home for three years (1).

Many people like E. R. who suffer from serious chronic insomnia rely on sleeping pills, and these medications have major problems. One is that using sleeping pills has been shown to increase the risk of dying during a 2 ½ year period by as much as 300% (2). Another is many people find sleeping pills can make them groggy the next morning, over time it can take larger and larger doses for the sleeping pills to work, and when they try to stop their insomnia comes back worse than it was before they started the sleeping pills.

Safer and more effective treatments for people with serious insomnia are clearly needed. This project will study the brain circuits that control being awake, being in dream sleep (rapid eye movement (REM) sleep), and being in non-dreaming sleep (non-REM sleep). The goal is to gain new information on how these circuits work so scientists can develop better treatments for people with chronic insomnia.

The proposed research cannot be done in a human clinical study for a number of reasons. First, the experiments involve surgical procedures, including the permanent placement of electrodes inside the brain. In addition, we need to obtain brain tissue to examine under the microscope after the sleep study is completed. Therefore, this research cannot be carried out as part of a human clinical study. It is also not possible to use human clinical pathology specimens because we first need to collect data from living cells, which is not possible with pathology specimens.

Cats are essential to this study for several reasons: a) Much of the previous work on the neuroscience of sleep has been carried out in the cat, so there is already a lot of information available to build upon, b) Cats become very comfortable with the researchers and laboratory environment, and typically spend about two thirds of their time sleeping in the lab, c) Cats have REM sleep (dreams occur during this sleep state) for long periods of time (up to 20 minutes) like humans do, while other

laboratory species like rats and mice have such short REM sleep periods that we cannot do the necessary studies on them.

The cats are given lots of attention and play time so they become very tame with the researchers and very comfortable in the laboratory, where they go to sleep naturally while we record their brain waves. Because we collect so much data from each cat, we only need six cats for this study. The surgery done to implant electrodes into their brains is the same surgery done for certain patients with Parkinson's disease, Essential Tremor, or epilepsy – the electrodes are just placed in different areas. Since the brain does not feel pain, the electrodes themselves cause no pain. The surgery is done under full anesthesia with post-operative pain relievers just like in human patents.

1) Deployment-related insomnia in military personnel and veterans.
Bramoweth AD, Germain A.
Curr Psychiatry Rep. 2013 Oct;15(10):401. doi: 10.1007/s11920-013-0401-4. Review.

2) Hypnotics' association with mortality or cancer: a matched cohort study.
Kripke DF, Langer RD, Kline LE.
BMJ Open. 2012 Feb 27;2(1):e000850. doi: 10.1136/bmjopen-2012-000850. Print 2012.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

As noted above, the cats are given lots of attention and play time so they become very tame with the researchers and very comfortable in the laboratory. Electrodes will be surgically implanted into their brains to record their brain waves and brain cell activity while they sleep. The surgery done to implant electrodes into their brains is the same surgery done for certain patients with Parkinson's disease, Essential Tremor, or epilepsy – the electrodes are just placed in different areas. Since the brain does not feel pain, the electrodes themselves cause no pain. The surgery is done under full anesthesia with post-operative pain relievers just like in human patients.

Animals will be given post-operative pain-killers for 3 days and then have a one-two week recovery from surgery. We will spend a lot of time handling and playing with these animals so they are very tame and comfortable in our laboratory set up. Five days a week for three to six weeks we will bring the animals to the laboratory where they will fall asleep naturally. We will record their sleep for 3 to 4 hrs and then feed them a treat and play with them for a while before returning them to their home cages.

Experiment 1: While they cats are sleeping, we will study the neurons in a brain area called the nucleus pontis oralis (NPO). Tiny amounts of drugs will be administered directly on to these neurons so we can learn how the NPO neurons respond to them. Learning how the NPO works in sleep will help direct future research into better drugs for insomnia.

Experiment 2: This experiment will focus on two brain areas, the NPO and the dorsal raphe nucleus (DRN). We will study how they interact during sleep and how different kinds of drugs affect this interaction. We think these two areas can work together to bring about sleep, and that understanding how different drugs affect how these brain areas work together will lead to better treatments for insomnia.

Once the experiments are completed, the animals will be painlessly euthanized with the same drug veterinarians use. Their brains taken and examined under the microscope to learn even more about connections between neurons in the NPO and DRN.

2. **Complete description of the proposed use of animals. (NOTE TO REVIEWERS: This section is similar but not exact to ACORP Version 3. Please note the new layout).**

Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

► **Experiments of this study are designed to elucidate the electrophysiological, functional and morphological bases for the key elements of the GABAergic Switching Network for Sleep and Wakefulness. Three sets of exepriments will be accomplished to acheave this goal. The first two studies will be performed in chronic cat preparations.**

The first set of experiments (Specific Aim 1) is aimed to identify *NPOGABAergic Wake-on neurons* that are responsible for promoting wakefulness and suppressing REM sleep. For this, in chronic cat preparations we will first record (intra/extracellularly) NPO neurons and identify GABAergic Wake-on neurons based on (i) the neurons' state-dependent discharge rate during naturally-occurring sleep and wakefulness and (ii) the cell's responses to the stimulations of the DR. NPO REM-on cells as well as other (non-GABAergic) Wake-on cells will be also identified. Then, we will determine electrophysiological properties of the recorded NPO neurons. Finally, we will identify neurotransmitter phenotypes and morphological profiles of these neurons by marking them intra/extracellularly with neurobiotin injection and by immunostaining.

Second set of experiments (Specific Aim 2), which will be also accomplished in chronic cats during naturally-occurring sleep and wakefulness, is aimed to (i) elucidate synaptic interactions and anatomical connections between the key neurons that constitute the GABAergic Switching Network for Sleep and Wakefulness, (ii) describe discharge patterns and membrane properties induced by various neurotransmitters (including GABA, glutamate, serotonin) with respect to morphologically identified neurons that comprise NPO GABAergic Wake-on neurons, NPO REM-on neurons and other types of identified cells in the NPO.

Studies will include the use of cats in recording, stimulation and morphological experiments using the chronic *in vivo* preparation. Adult cats weighing between 3.5-5.0 kg will be employed. Briefly, under isoflurane anesthesia, using sterile surgical procedures, cats will be implanted with electrodes for recording the electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG). A Winchester plug, connected to these implanted electrodes, and a chronic head-restraining device will be bonded to the skull with acrylic cement. A hole 4-5 mm in diameter will be placed in the calvarium overlying the cerebellar cortex for subsequent insertion of electrodes for recording or stimulating. A chamber which has a surrounding protective wall around the hole with sterilized acrylic cement will be built for the protection of the hole. The hole will be sealed and protected with a stainless or plastic screw closure (cap) which can be screwed on and off of a "finish" on the top of the chamber. Following surgery, triple antibiotic ointment around and inside of the hole will be topically applied, and antibiotics and analgesia will be administered. After recovery from surgery, all cats will be adapted to the recording conditions each day for two weeks. Thereafter, whenever they are placed in the recording apparatus, the animals will normally exhibit spontaneous periods of wakefulness, NREM and REM sleep. Experiments will be undertaken in these chronically-prepared cats during naturally-occurring states of sleep and wakefulness.

Each animal will first be used in Experiment 1, then at least 2 weeks later in Experiment 2.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

▶ **The basic plan for the statistical analysis of data is a 2 x 2 ANOVA design. Factor A has two levels (control and experimental) while factor B has two levels (control and the effect of a single drug). This basic design will be used to examine the effect of several drugs. With 16 neurons per treatment group, 64 neurons are required for each drug tested. This design achieves 80% power for an overall F-test at a 5% significance level when at least one mean is different by 0.5 SD. The assumption is we will be able to record each neuron under control conditions as well as following the successive application of more than one drug or condition. Since more than one neuron per animal will be studied, a repeated measures ANOVA may be required which will include a random effect for animal. Depending on the within-animal correlation, the power may be somewhat lower than 80%.**

We anticipate that recent advances in neurophysiological and immunohistochemistry techniques will enable us to record enough neurons from two animals to meet the needs of this study. If we are unable to record enough neurons from two animals, we will file a modification for one or more additional animals.

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
Describe each procedure in a few words or just one sentence, and then write "see appendix X for details". e.g. stereotaxic surgery – see appendix 5 for details, Morris water maze – see appendix 6 for details.

(NOTE TO REVIEWERS: This section is a significant change in the previous ACORP. Note that *departures from the Guide* are to be listed here and in appendix 9. This section will likely require significantly more attention from the VMO to identify departures from the GUIDE.

▶ **Procedure 1: survival surgery for chronic experiment (see Appendix 5 for details).**

Procedure 2: sleep recording in chronic cats (see Appendix 6 for details).

Procedure 3: electrical stimulation: A bipolar tungsten stimulating electrode will be employed to the DRN (the stereotaxic coordinates: AP 0 to -1.0, L 0, H 0 to 1.0) to deliver electrical stimulation. The purpose of the stimulation of the DRN is to antidromically activate NPO GABAergic neurons that project to the DRN by electrical stimulation of the axons or terminals of these neurons via a metal electrode consisting of a single stimulus (intensity: a few to several tens of μA; duration: 0.2 ms). In addition, the intensity of the stimulation will be slowly increased from zero to a few tens of μA to obtain waking threshold for the stimulating site, but stimulation intensity will be kept under the waking threshold during the whole period of experiments. For such a low intensity and short duration, and the above procedure of stimulation by gradually increasing stimulating intensity and keeping the intensity under the waking threshold, we will make every effort to ensure our animals will not suffer from the pain with this particular stimulation. Finally, we will be constantly monitoring the behavior of the animal during experiments. If there was the presence of signs of pain and discomfort, such as behavioral agitation (EEG desynchrony and maximal EMG activity, failure to fall asleep), the vocalization and excessive body movements, etc, the experiment would be immediately terminated and the animal would be returned to vivarium housing. The animal will be continuously monitored for recovery until it is calm.

D. **Species.**Justify the choice of species for this protocol.

These studies will be done in chronic cat preparations.

► Chronic *in vivo cat* preparation will be used in Experiments 1 and 2 - recording, stimulation and morphological experiments. Cats are essential to this proposal in several reasons. First, much of the previous studies dealing with the electrophysiology and neuropharmacology of the behavioral states of sleep and wakefulness have been carried out in the cat. Thus, there is a large database, including our recent findings that GABAergic inhibitory processes in the NPO play a critical role in the control of REM sleep and wakefulness in the cat that is available to evaluate and compare with the results of the present proposal. Second, cats adapt readily to the laboratory environment, where they spend about two third of their time on sleep (REM sleep and NREM sleep). Most importantly, cats have long duration episodes of REM sleep compared to rats or mice. REM sleep episodes can last up to 20 min in the cat, permitting the reliable measurement of pharmacological effects on NPO cells during this state. On the other hand, REM sleep episodes last only a few minutes in the rat or mouse, making such measurements impossible. Third, the large brain of the cat makes it ideal for us to carry out the preceding experiments that involve prolonged intracellular recordings over an hour in duration in conjunction with the simultaneous microiontophoretic/pressure application of neurotransmitter agonists and antagonists. It is also possible to implant electrodes for recording EEG, EOG, EMG and PGO activity and to use multi-barrel micropipettes for studying the effects of drugs on REM sleep and wakefulness. Such experiments are almost impossible to carry out in rats or mice. Finally, chronic cat preparations allow us to use the same animal for multiple experiments in an intensive, within-subjects, experimental design. Thus, the number of animals used in our studies can be minimized.

Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. PI
   Name►[redacted] **Ph.D.**
   Animal research experience ►**Dr.[redacted]has been conducting animal research for over 30 years and has dealt with all aspects of the experiments that are described in this protocol.**

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Oversee experimental design, data analysis and manuscript preparation.	**Dr.[redacted]will oversee the planning, development and execution of this research project at the VMU GLAHS. [redacted]extensive experience in studies utilizing the cat as an animal model make[redacted]an ideal candidate for the determining the proper scientific approach and technique.**
Chronic cat surgeries, data handling, and research publication.	**Dr.[redacted]has been performing survival surgeries and running experiments pertaining to this protocol for over 20 years.[redacted]is the first author on numerous articles in peer-reviewed publications that deal with findings in chronic cat preparations.**

Electrophysiological recording from awake and sleeping cats	**Dr. [redacted] has performed these kinds of recordings in cats for over 20 years.**

Co-PI
Name► [redacted] **PhD**
Animal research experience ► **Over 35 years of conducting animal research and experience of doing the procedures on this ACORP. Dr. [redacted] will assist Dr. [redacted] in the overall planning, organization and performance of this research.**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
	The co-PI will have no animal contact.

1. Other research personnel (copy the lines below for each individual)

Name► [redacted] **PhD**

Animal research experience ► **Dr. [redacted] has been conducting animal research for over 30 years. [redacted] is an expert in carrying out experiments pertaining to this protocol.**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Chronic cat surgeries.	**Dr. [redacted] has performed these surgeries on cats for over 30 years. [redacted] and Dr. [redacted] are the first to successfully obtain intracellular and extracellular recordings from hypoglossal motoneurons in chronic cats ([redacted] 2014, 2015).**
Electrophysiological recording from awake and sleeping cats.	**Dr. [redacted] has performed these kinds of recordings in cats for over 30 years.**

Name► [redacted] BS

Animal research experience ► [redacted] was a Laboratory Assistant at [redacted] in 2015. [redacted] assisted the Senior Researchers with previous projects and has experience with similar experiment protocols.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP

Acute rodent surgeries and data handling	[redacted]has assisted in non-survival surgeries and running experiments pertaining to this protocol.

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)
   Name► **To be determined by VMO (NOTE TO REVIEWERS: This should always be "TBD BY VMO) and will be pre-populated)**

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
TBD BY VMO	TBD BY VMO

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course. **(PI must fill it out after consultation with Dr. John Berard, IACUC coordinator)**

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted]	12/20/17	9/17/17 (cat)	
[redacted]	3/17/2018	3/9/2018 (cat)	
[redacted]	7/20/2017	7/20/2017 (cat)	
[redacted]	1/17/2018	1/16/2018 (cat)	

F. **Training to be provided.** List here each procedure in E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, check box "N/A"
► (X) **N/A**
► Additional training:

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

Name	Enrollment in Occupational Health and Safety Program		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
[redacted]	(x)			Yes

[redacted]	(x)			Yes
[redacted]	(x)			yes
[redacted]	(x)			Yes

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

► ( ) Yes. Describe them ►

► (X) No.

Animals Requested
(NOTE TO REVIEWERS: This section is basically the same as previous version of the ACORP)

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Cat	**Male and Female**	1-3 yrs of age	[redacted] now known as [redacted]	Normal/healthy

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures►						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category B TOTAL**

USDA Category C

Procedures►						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category C TOTAL**

USDA Category D

Procedures▶ Surgery/restraint/recording						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category D TOTAL**
Cat/each cat serves as both pre-drug control and post-drug treated groups/ survival surgeries	**2**	**2**	**2**			**6**

USDA Category E

Procedures▶						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category E TOTAL**

TOTALS over all Categories

Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**GRAND TOTAL**
Cat/each cat serves as both pre-drug control and post-drug treated groups/ survival surgeries	**2**	**2**	**2**			**6**

J. **Management of USDA Category D procedures.** Indicate which statement below applies, and provide the information requested.

▶ ( ) This protocol does NOT include any Category D procedures.

▶ (X) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

Procedure	Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for the monitoring	Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered)

Survival surgery for implanting chronic electrodes (See Appendix 5 for details)	**Check every 5 minutes for vital signs (core temperature, heart and breathing rate). We also check for depth of anesthesia by checking changes in heart/ breathing rate and withdrawal reflex in response to toe-pinch**	**Veterinarian and veterinarian technicians, as well as Dr. [redacted] and Dr [redacted]** **Anesthesia assistance during the surgery will be arranged in advance with the VMU.**	Prior to surgery, these injectable drugs are given once: "DKT" aka Kitty Magic (0.1 ml Dexmedetomidine, 0.1 ml Ketamine, 0.1 ml Butorphanol) i.m., Carprofen 5 mg/kg, s.c., dexamethasone, 0.5 mg/kg, i.m.; atropine sulfate, 0.04 mg/kg, i.m.; Buprenorphine, 0.02 mg/kg s.c. Anesthetic: Isoflurane, 2-4%, inhalation via tracheal intubation, throughout the surgery. Postoperation Atipamezole (0.1 ml) i.m. to reverse DKT, analgesic: Buprenorphine, 0.02 mg/kg s.c., every 12 hours, for 3 days; Meloxicam 5 mg/kg, s.c., once daily for 3 days; Antibiotic: Baytril 2.27%, 0.11 ml/kg, s.c., once daily for 3 days.
Re-suture post-surgery	Check every 15 minutes for vital signs (heart and SPO2). We also check for depth of anesthesia by checking changes in heart/ breathing rate and withdrawal reflex in response to toe-pinch	Dr. [redacted] Dr. [redacted]	Anesthetic: Isoflurane, 2-4%, inhalation, throughout surgery; lidocaine applied locally to the procedure site

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

▶ (X) This protocol does NOT include any Category E procedures

▶ ( ) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.

▶

Veterinary Care and Husbandry

L. **Veterinary Support.**

1. The laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

   (NOTE TO REVIEWERS: This section will be prepopulated)
   Dr. [redacted], DVM,
   VMO, GLAHS
   [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted▶ **Dr. [redacted], DVM,**

   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ▶ **04/07/2016 and 06/13/2016**
   ▶ **09/25/19**

M. **Husbandry.** As a reference for the animal husbandry staff, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.) **(NOTE TO REVIEWERS: This is a potentially confusing section that is a significant departure from previous ACORPs. Please be aware of non-standard housing and *departures from the GUIDE*. The VMO should provide extra attention to this section. Areas that may be important in this section are housing of breeding pairs, weaning of babies, and housing of animals singly for experimental reasons.)**

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

a. Species	b. Type of housing*		c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)		e. Estimated maximum number of housing units needed at any one time
Cat	X	**Standard**	TBD by VMO	X	**Yes**	2
		Departures from the Guide			**No**	

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:

▶ (X) **Standard (See SOP)—Enter SOP in the table in Item Y.**
▶ ( ) **Standard (not covered by a SOP)**
▶ **Describe:**

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a

justification if any animals will be housed singly (if species is not considered "social", then so note)

► (X) **N/A: Animals will be housed in stable pairs or groups .**
► ( ) Animals will be housed singly:
►Provide justification:

*****Use Appendix 9 to document "departures" from the standards in the *Guide*.**

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

(NOTE TO REVIEWERS: This section will be prepopulated, as the VMO will determine appropriate enrichment)

a. Species		b. Description of Enrichment*		c. Frequency	
cat	X	**TBD By VMO**		X	**TBD By VMO**
		Non-standard enrichment (describe and justify below)			**Other**

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here. **(NOTE TO REVIEWERS: In those cases where experimental design or other factors preclude use of standard enrichment, the PI may need to fill out the "non-standard box").**

► (X) **Standard (TBD by VMO)**
► ( ) **Non-standard**
Description on non-standard enrichment and justification:

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.

►

► ( ) This ACORP does NOT include use of any animals that will require customized routine husbandry. If checked, go to item N.

► (X) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.

► ( ) **N/A**
► (X) **Describe: The marginal surgical areas will be cleaned according to procedures requested by Dr. [redacted] D.V.M. and/or by using chlorehexedine as follows: On a routine basis, the marginal area surrounding the cranial implant will be cleaned with chlorehexedine. The surrounding area will first be moistened using a swab (Q-tip, cotton, or gauze) that contains chlorehexedine. Once the area is moist, a Q-tip containing chlorehexidine is used, a section at a time, to remove dead tissue. Dead tissue that is hard to remove with the Q-tip is removed with a forcep. <u>After chlorhexidine swab cleaning, chlorhexidine residue will be removed with sterile saline and gently dried with gauze.</u>**

► (**X**) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.

► ( ) **N/A**
► (**X**) **Describe: The plexiglass cages will be scrubbed with betadine and washed and rinsed weekly.**

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed. **(NOTE TO REVIEWERS: All VMU space should be noted as TBD BY VMO.)**

► (X) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU. If it will be in the VMU, just indicate West LA VMU or Sepulveda VMU.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]VMU	TBD by VMO	X	

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table. **(NOTE TO REVIEWERS: This space is for NON VA Facilities, like [redacted] and [redacted]. Dr. [redacted] can assist on AAALAC status).**

Name of Non-VA Facility	Is this facility accredited by AAALAC?		Building	Room Number
	Yes -- enter status*	No**		
		( )**		
		( )**		
		( )**		

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".
► (X) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (X) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".
► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

Procedure	Surgical?		Bldg/Room Number	Requires transport between the VMU and the laboratory, or transport between laboratories?	
	Yes	No		No	If Yes – describe method of discreet transport
head electrode implant	(x)	( )	VMU operating room	(x)	
Polygraphic , intracellular and EEG/EMG recordings in conjunction with microinjections of neurotransmitter agonists and antagonists	( )	(x)	**Bldg [redacted] Room [redacted]**	( )	
Intracardiac perfusion with fixative		X	**VMU Bld. [redacted] Necropsy room (Room [redacted]**		Animal will be transported in a pet carrying case covered with a drape.
Re-suture post-operation	X		[redacted]		Animal will be transported in a pet carrying case covered with a drape.

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

Body Fluid, Tissue, or Device to be Collected	Collected AFTER Euthanasia	Collected BEFORE Euthanasia		
		Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
Brain	X			

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► (X) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".
► ( ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved).

In addition, specify how often the animals will be weighed to be sure weight loss does not exceed 10%.

(NOTE TO REVIEWERS: This section is important and should be carefully reviewed to ensure humane and safe endpoint criteria. The VMO should spend particular attention to this section as well. Departures from the Guide should be noted as described in Appendix 9).

► continual depression, loss of appetite, significant weight loss (over 10%), aggression, neurological signs (abnormal gait, cerebellar signs of motor deficit (e.g., ataxia and dysmetria), lethargy), abnormal phase-switching of sleep-wake cycles, failure to groom, illness refractory to veterinary intervention, wound dehiscence, and dislodgement of head implant.

U. **Termination or removal from the protocol**. Complete each of the following that applies:

► ( ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

► (X) Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures

from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death▶ Method for verifying death▶ Secondary physical method▶				
X	Anesthetic overdose Agent▶ Acepromazine Dose▶0.1 mg/kg Route of administration▶ s.c. Agent▶ Pentobarbital Dose▶100 mg/kg Route of administration▶ i.v. Method for verifying death▶ a lack of heartbeat, follow by thoracotomy and perfusion	cat	X		
	Decapitation under anesthesia Agent▶ Dose▶ Route of administration▶				
	Exsanguination under anesthesia Agent▶ Dose▶ Route of administration▶ Method for verifying death▶				
	Other (Describe) ▶ Method for verifying death▶				

	Other (Describe) ► Method for verifying death►				

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:

   ► (X) **N/A**
   ► ( ) **Justification:**

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:

   ► (X) **N/A**
   ► ( ) **Justification:**

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.

   ► ( ) **N/A**
   ► (X) **Justification:** Drs. [redacted] and [redacted] will perform euthanasia. They have more than 20 years of experience for carrying out these procedures in this animal specie.

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
   (NOTE TO REVIEWERS: The VMU has a specific SOP (The Biocontainment SOP). Normally, this SOP would be the one used in this section. Only if some other SPECIFIC alternative procedure is used would the other box be checked.

   ► (X) **According to Biocontainment SOP.**
   ► ( ) **Not according to Biocontainment SOP:**
   ►**Justification and description (must review first with VMO):**

   b. Describe how the PI's staff should be contacted.

   ► (X) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
   Name► Dr [redacted]
   Contact Information► [redacted] office), [redacted] (VA lab); [redacted] (cell phone); email: [redacted]

▶ ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
▶

Name▶
Contact Information▶

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

(NOTE TO REVIEWERS: This is a confusing section to understand and may require some additional discussion and review as to what special procedures should be listed here—but may generally include things like diet/fasting, restraint, etc).

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP – specify the Item letter(s)	Appendix 6
Electrophysiology recording in sleeping cats		Items:	(X) **
head restraint conditioning		Items:	(X) **
		Items:	( ) **
		Items:	( ) **

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

(NOTE TO REVIEWERS: This new section is slightly modified from previous ACORP and focuses on replacement/refinement/reduction).

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   - ► ( ) **N/A**
   - ► (X) **Painful or distressing procedures:**

     ► Having chronic head electrodes implanted.
     Restraint for sleep recording from intracellular electrodes.

   Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.
   PI should run at least one search on the ALTBIB website for animal use alternatives. Please use the link http://toxnet.nlm.nih.gov/altbib.html

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
PubMed	10-17-19	All available years to present	N/A.	dorsal raphe nucleus, GABA, REM ***26 items found***	( )	( )	( )	(x)
PubMed	09-26-19	2000-present	N/A.	"nucleus pontis oralis, GABA, REM " ***14 items found***	( )	( )	( )	(x)
PubMed using ALTBIB animal alternatives search strategy	10/25/19	All available years to present	N/A.	sleep and (nucleus pontis oralis OR dorsal raphe nucleus) ***No items found***	( )	( )	( )	(x)
PubMed using ALTBIB animal alternatives search strategy	09-26-19	2000-present	Having chronic electrodes implanted into the NPO/brainstem.	"head restraint", "chronic intracellular recording", " nucleus pontis oralis" ***No items found***	(x)	(x)	(x)	( )

Go3R Web	09-26-19	2000-present	Having chronic electrodes implanted into NPO/brainstem.	"head restraint", "chronic intracellular recording", " nucleus pontis oralis, REM" ***No items found***	(x)	(x)	(x)	( )
Education Resources Information Center (ERIC)	09-26-19	2000-present	Having chronic electrodes implanted into NPO/brainstem.	"head restraint", "chronic intracellular recording", " nucleus pontis oralis, REM" ***No items found***	(x)	(x)	(x)	( )

2.

Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.

►Cats are essential to electrophysiological and pharmacological studies of this protocol in several reasons. First, much of the previous studies dealing with the electrophysiology and neuropharmacology of the behavioral states of sleep and wakefulness have been carried out in the cat. Thus, there is a large database, including our recent findings that GABAergic inhibitory processes in the NPO play a critical role in the control of REM sleep and wakefulness in the cat that is available to evaluate and compare with the results of the present proposal. Second, cats adapt readily to the laboratory environment, where they spend about two third of their time on sleep (REM sleep and NREM sleep). Most importantly, cats have long duration episodes of REM sleep compared to rats or mice. REM sleep episodes can last up to 20 min in the cat, permitting the reliable measurement of pharmacological effects on NPO cells during this state. On the other hand, REM sleep episodes last only a few minutes in the rat or mouse, making such measurements impossible. Third, the large brain of the cat makes it ideal for us to carry out the preceding experiments that involve prolonged intracellular recordings over an hour in duration in conjunction with the simultaneous microiontophoretic/pressure application of neurotransmitter agonists and antagonists. It is also possible to implant electrodes for recording EEG, EOG, EMG and PGO activity and to use multi-barrel micropipettes for studying the effects of drugs on REM sleep and wakefulness. Such experiments are almost impossible to carry out in rats or mice. Finally, chronic cat preparations allow us to use the same animal for multiple experiments in an intensive, within-subjects, experimental design. Thus, the number of animals used in our studies can be minimized. For the preceding reasons, we firmly believe that cats, not rats or mice are the animals of choice for our in vivo chronic studies described in the ACORP.

Since non-mammalians do not have REM sleep, and the brain structures of these animals are significantly different from mammalians, therefore, there is no scientific merit for us to carry out our proposed research using non-mammalian models (Note that we are aware of the reports that some non-mammalian species might experience REM sleep, but these findings are still questionable).

Computer models have been considered, but literature searches do not indicate that they can replace those methods described in this protocol because there are no alternatives that provide data related to the normal behaviors of sleep and wakefulness. There are insufficient data that describe the activity of GABAergic neurons in the NPO and how they discharge during sleep and wakefulness to be able to develop a model to investigate. Therefore, computer models could replace none of the animal procedures described in the ACORP.

Though in vitro (brain slice) study is a possibility but this reduced preparation typically transects all brain input pathways to the target neurons of interest. Importantly, it is impossible to induce sleep and waking states and in vitro models lack standard correlative indices of REM sleep such as polygraphic (EEG) changes and motor/EMG atonia. Therefore, this preparation lacks the physiological significance, i.e., state-dependency of data collected from such studies.

As can be seen above, we did extensive literature searches on a number of animal use alternative websites for suitable alternatives and found no papers.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.

► **Based upon our past experience in chronic cat studies, we estimated an average total of 2 cats per year will be minimally required for harvesting meaningful scientific data for our proposed studies. Each cat serves its own control (pre-drug) as well as treatment (post-drug) measurements. Therefore, the group size needed for the control and the treatment groups are effectively reduced. To maximize the amount of data collected from each chronic cat, we will acquire data on multiple days over the viable period (approximately 4 weeks) of the implanted nerve cuff electrodes. These electrodes are known to have a limited duration of working life, due to unavoidable scar formation around the implants which ruins the recording and stimulating capability of the electrodes. To further optimize the experimental design, we will perform the acute (carbachol paradigm) study on the terminal day of a given chronically instrumented cat, instead of using a new cat. The carbachol paradigm is a standard experimental procedure of pharmacologically-induced REM sleep which is widely used in sleep research field. In this experimental session, carbachol (0.25 µl , 22 mM in saline), a cholinergic agonist, is microinjected into the dorsolateral part of the rostral pontine reticular formation of the animal to induce, with a short latency, a prolonged episode of REM sleep (>60 min).**

We calculated the minimal number of animals we need in order to get enough neurons recorded from – see section C2b for details.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.

► **We will adopt the following steps for refinement of our studies.**

Proper pre- and post-operation procedures are strictly followed in order to lessen or eliminate pain or distress, and infection. Veterinarian care will be provided by the WLA VMU staffs. Best appropriate use of analgesics, anesthetics, and tranquilizers will be adopted according to recommendations of the veterinary medical officer. We were given a recommendation from Dr. [redacted] to replace xylazine with the DKT mixture aka Kitty Magic and to replace carprofen with meloxicam. Both replacements are more modern techniques used to keep the cats in better health.

Softened food will be given to the cats over the first few days post-surgery, since they may have some discomfort with swallowing at that time.

Animals will be played with extensively to make them comfortable with the researchers and the laboratory before we start doing any recordings. After each recording session, animals will be given canned food as a treat, and played with some more to give them exercise and pleasure.

Humane endpoints will be used so animals are euthanized if they show signs of weight loss (10%), lack of grooming, cerebellar signs of motor deficits, or abnormal sleep-wake cycles. By feeding them moist food as a treat after each recording session, we will be able to see if they are having any problems chewing or swallowing (since these are controlled by the hypoglossal nucleus). We also will play with them with a laser pointer after each session which lets us see if there are any neurological deficits (problems with visual tracking, or motor problems affecting coordination in activities such as chasing and pouncing).

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.

►Literature searches indicate that none of the proposed studies have been conducted previously and the data that we seek to obtain are new and unique. There are only a few groups working in this area, and no other group anywhere has been able to do chronic intracellular recordings. Many of the papers we brought up come from our group, and we are building on that earlier work.

X. **Other Regulatory Considerations.**

1. **Controlled drugs.**

► ( ) **N/A (Go to Question 2).**
► (X) Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Pharmacy	Non-VA
Ketamine	X	( )*	TBD	X		X	
Pentobarbital	X	( )*	TBD	X		X	
Buprenorphine	X	( )*	TBD	X		X	
Butorphanol	x	( )*		x		x	

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.

► (X) **N/A**
► ( ) **Justification:**

a. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

►( ) **N/A**
►(X) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.
►( ) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.
►( ) Other. Explain►

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

►( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".
►(X) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

►( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".
►(X) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

►(X) Appendix 1, "Additional Local Information"
►( ) Appendix 2, "Antibody Production"
►(X) Appendix 3, "Biosafety"
►( ) Appendix 4, "Ante-mortem Specimen Collection"
►(X) Appendix 5, "Surgery"
►(X) Appendix 6, "Special Husbandry and Procedures"
►( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
►( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
►( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"
►( ) Appendix 10, "Overnight housing"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

(NOTE TO REVIEWERS: This section will have to be developed over time. Some of the information will be pre-populated).

Item	SOP		Approval Date
	Title	ID	

C.2.c	This needs to be pre-populated		
M.1			
M.2			
U.4.a			
U.4.b			
V			

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

   a. **Certification by Principal Investigator(s):**

   I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

   I understand that further IACUC approval must be secured before any of the following may be implemented:

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
   - Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
   - Performing any additional procedures not already described in this ACORP;
   - Use of any of these animals on other protocols, or by other investigators.

   I further certify that:

   - No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
   - I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Principal Investigator	PI Signature	Date
[redacted] PhD.	[redacted]	09/17/19

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

▶ ( ) No minority opinions were submitted by any IACUC participant for inclusion.

▶ ( ) Minority opinions submitted by IACUC participants are copied here ▶

▶ ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages▶)

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted] **MA, DVM**	[redacted]	10/29/19
Name of IACUC Chair		Date
[redacted] **PhD**	[redacted]	10/29/19

2. **Appendix 2. Antibody Production.** No signatures required.

3. **Appendix 3. Biosafety.**

a. **Certification by PI(s) and IACUC Officials:**

We certify that:

- Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

- All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Principal Investigator	Signature	Date
[redacted] **PhD.**	[redacted]	09/17/19
Name of Institutional Veterinarian	Signature	Date
[redacted] **MA, DVM**	[redacted]	10/29/19
Name of IACUC Chair		Date
[redacted] **PhD**	[redacted]	10/29/19

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";
- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee	Signature	Date
[redacted] **PhD**	[redacted]	10/17/19

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";

- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee	Signature	Date

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:
  - Identification of each animal such that care for individual animals can be documented.
  - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
  - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
  - Daily records covering at least the period defined as "post-operative" by local policy.
  - The signature or initials of the person making each entry.

Principal Investigator	PI Signature	Date
[redacted] **PhD.**	[redacted]	09/17/19

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

   a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

Principal Investigator	PI Signature	Date

   b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of the Manager of the Human Patient Care Equipment	Signature	Date

   c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date

Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of Chief of Staff	Signature	Date
Name of Director or CEO of the Facility (Hospital or Clinic)	Signature	Date

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

   a. **Certification by the Principal Investigator(s).**

   I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

   I further certify that:

   - Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
   - All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
   - Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
   - Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

Principal Investigator	PI Signature	Date

b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of VISN Regional Safety Officer	Signature	Date

9. **Appendix 9. Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

10. **Appendix 10. Certification by Principal Investigator is on the Appendix.**

[redacted]
05006-18▶
Official Date of Approval▶

ACORP Appendix 1
ADDITIONAL LOCAL INFORMATION
VERSION 4 V2 6/17/2015
(Required for all protocols)

(See ACORP App. 1 Instructions, for more detailed explanations of the information requested.)

Species covered by this Appendix: **Cat**

This protocol involves the following (check all that apply):

☐ Breeding ☐ Tumor Formation ☒ Hazardous agents used in animals
☐ Multiple survival surgery ☐ Food and/or Fluid Restriction
☐ Antibody/Ascites Formation ☐ Hazard to VMU Personnel
☒ Prolonged Restraint (> than 15 minutes) ☐ Tumor formation

a.VA project # [redacted]	b. Protocol # **05006-18**	c. 3 year expiration date **10/31/2019**
d. PI name: [redacted] **PhD**		e. PI phone: [redacted]
f. PI e-mail: [redacted]		g. Species: **cat**
h.Protocol title: **GABAergic Switches Control Wakefulness, NREM sleep and REM sleep**		
i. Contact name 1: [redacted] **, PhD**		j. e-mail: [redacted]
k.Contact name 2: [redacted]		l. e-mail: [redacted]
m. Lab phone: [redacted]	n. Alternate phone: [redacted]	o. **EMERGENCY PHONE # (Cell preferred)** 1- ([redacted]

p. Animals taken to lab? ☒Yes ☐ No If yes, Bldg: [redacted] and Room(s) [redacted]

q. Animals taken to lab and then returned to vivarium (*VMU return room only*) ☒Yes ☐ No If yes, provide a scientific justification here: We conduct *in vivo* recordings in the chronic cat preparation.

r. Animals housed in the lab for 12 or more consecutive hours? ☐ Yes ☒ No
If yes, Bldg: and Room(s): and fill out part B below.

s. Is wire-floored caging required? ☐ Yes ☒ No

t. Do animals need to be exempted from the environmental enrichment program?
☐ Yes ☒ No If yes, provide a scientific justification here:

u. Maximum allowable body weight loss (10% unless scientifically justified): **10%**

v. Hazards used in animals (check all that apply): ☐ None ☒ Toxic ☐ Infectious
☐ Biological ☐ Radioactive ☐ Other (list):

w. Will VMU personnel be exposed to any of these hazards? (This includes animals housed in labs since VMU staff check them, wash the cages, etc.) ☐ Yes **X** No
If yes, list which hazards:

x. Body fluid, tissue and/or device collection? ☐ None ☐ Live ☒ Dead ☐ Both

y. Surgery? ☐ None ☒ Minor ☒ Major ☒ Both ☒ Non-survival
Multiple survival surgeries? ☐ Yes ☒ No If there are multiple survival surgeries, list surgery types: **Head electrode implant; re-suture post-surgery**

z. Anesthetics/analgesics used (excluding euthanasia)? ☒ Yes ☐ No If yes, list: Ketamine, carprofen, isoflurane, buprenorphine, lidocaine, xylazine, butorphanol, dexmedetomidine, meloxicam.
aa. Euthanasia methods (must include anesthetic plus physical method unless scientifically justified): Pentobarbital iv
bb. All controlled substances used: buprenorphine, ketamine, Pentobarbital, Butorphanol
cc. List any other drugs from Appendix 5 (surgery appendix): xylazine, baytril, atropine sulphate, dexamethasone, atipamezole.

Delegation of Authority: Complete this section for every employee in this study, starting with the PI, specifying which procedures each is allowed to perform. All should be listed in the ACORP main body. Everyone listed must also have current employment status (VA or WOC) and be up-to-date with all required training and medical clearances.

Please note: There must always be at least one person responsible for task codes A, D, and H.

Species :

Last name, first name, degree(s):	Task codes (use the list below):
[redacted] **PhD**	**A,B,C,D,G,H,I,J**
[redacted] **PhD**	**A,B,C,D,G,H,I,J**
[redacted] **BS**	**A ,D,G,I,J**
[redacted] **PhD**	**J**

Task codes

A = Routine daily care of animal B = Performs survival surgery C = Performs non-survival surgery	I = Performs in vivo procedures other than sample collection or surgery, such as behavioral studies J = Other work with animals, please specify: **data analysis**

D = Evaluates endpoint criteria E = Collects samples with anesthesia F = Collects samples without anesthesia G = Collects or works with samples postmortem H = Euthanizes animal subjects	K = PI with no animal contact. The PI must be listed in section E of the ACORP Main Body. If the PI does have animal contact, list them with the appropriate task codes. L = Non-PI with no animal contact. This person does not need to be listed in section E of the ACORP.

[redacted]
05006-18▶
Official Date of Approval▶

ACORP APPENDIX 3
BIOSAFETY
VERSION 4 V2 6-17-2015

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

Material Identify the specific materials including radioisotopes, chemicals, drugs (standard clinical agents as well as test agents), controlled substances, infectious agents, biomaterials, prosthetic devices, minipumps, special diets, and cells, tissues, or body fluids. *(Do not list drinking water and the standard food from the VMU)*	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**							
		Toxic Agent (such as mutagens, carcinogens, teratogens, neurotoxins, Select Agents, ect. -Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, or 2*)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent	Other
formaldehyde	Fisher Scientific	(x)	( )BSL_	( )	( )	( )	( )	( )	( )
GABA (γ-Aminobutyric acid)	Sigma-Aldrich								x
Bicuculline ([R-(R*,S*)]-6-(5,6,7,8-Tetrahydro-6-methyl-1,3-dioxolo[4,5-g]isoquinolin-5-yl)furo[3,4-e]-1,3-benzodioxol-8(6H)-one)	Sigma-Aldrich	x							
Phaclofen (3-Amino-2-(4-chlorophenyl)propylphosphonic acid)	MP Biomedicals	(x)	( )BSL_	( )	( )	( )	( )	( )	( )
Muscimol (5-Aminomethyl-3-hydroxyisoxazole)	Tocris Bioscience	(x)	( )BSL_	( )	( )	( )	( )	( )	( )
Baclofen (4-Amino-3-(4-chlorophenyl)butanoic acid)	Tocris Bioscience	x							

Serotonin (3-(2-Aminoethyl)-5-hydroxyindole hydrochloride)	Sigma-Aldrich	x							
glutamate	Sigma-Aldrich								x
Pindolol (1-(1H-Indol-4-yloxy)-3-(isopropylamino)-2-propanol)	Tocris Bioscience	x							
Ach (Acetylcholine)	Sigma-Aldrich	x							
8-OH-DPAT (8-Hydroxy-2-(dipropylamino)tetralin)	Sigma-Aldrich								x
Muscimol-conjugated Magnetonanoparticles	Corpuscular Inc.	x	( )BSL_						
Neurobiotin (N-(2-aminoethyl) biotinamide hydrochloride)	Vector laboratories								x
PHA-L (Phaseolus vulgaris agglutinin)	Vector laboratories								x
CtB (Cholera Toxin B subunit)	Sigma-Aldrich								x
isoflurane	VMU pharmacy						x		
lidocaine	VA pharmacy						x		
xylazine	VA pharmacy						x		
buprenorphine	VA pharmacy						x		
baytril	VA pharmacy						x		
atropine sulphate	VA pharmacy						x		
dexamethasone	VA pharmacy						x		
ketamine	VA pharmacy						x		
saline	VA pharmacy						x		
Carprofen	VA pharmacy						x		
carbachol	Sigma-Aldrich	x							
Pentobarbital	VA pharmacy	x					x	x	
Ophthalmic ointment	VA pharmacy						x		
betadine	VA pharmacy						x		
Rubbing alcohol	VA pharmacy						x		
Meloxicam	VMU pharmacy						x		

Dexmedetomidine	VMU pharmacy						x		
Butorphanol	VMU pharmacy						x		
Atipamezole	VMU pharmacy						x		

Only BSL 1, 2 or 2* work is permitted at VA-GLA. No BSL 3 or 4 work.

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in Item 1 above. For each item, note if it is USP grade, FDA approved, a fixative, or a special diet.

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify "Main Body" or "App #", and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)
Formaldehyde*	10%, 2000ml	Sterile saline	intracardiac	Once, 30 minutes	Fixative, brain will be fixed for histology	ACORP main body, item C2	yes
GABA*	200mM, $0.2x10^{-3}$ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	Endogenous GABAergic neurotransmitter; neuron excitability decreases	ACORP main body, item C2	No
Bicuculline*	1mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$GABA_A$ receptor antagonist; neuron excitability increases	ACORP main body, item C2	No
Phaclofen*	0.5mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$GABA_B$ receptor antagonist; neuron excitability increases	ACORP main body, item C2	No
Muscimol*	5 mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$GABA_A$ receptor agonist; neuron excitability decreases	ACORP main body, item C2	No

baclofen*	10 mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$GABA_B$ receptor agonist; neuron excitability decreases	ACORP main body, item C2	No
Glutamate*	200mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	Endogenous Glutamate neurotransmitter; neuron excitability increases	ACORP main body, item C2	No
Serotonin*	10mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	Endogenous monoamine neurotransmitter; neuron excitability increases	ACORP main body, item C2	No
8-OH-DPAT*	1mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$5\text{-}HT_{1A}$ receptor agonist; neuron activity decreases	ACORP main body, item C2	No
Pindolol*	1mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	$5\text{-}HT_{1A}$ receptor antagonist; serotonergic *neurons* activity decreases	ACORP main body, item C2	No
ACh*	1mM, $0.2x10^{-3}$ ml	Sterile saline	iontophoresis, pressure ejection	Multiple (2-3) trials, 3-10 minutes	Endogenous Cholinergic nuerotransmitter; neuron excitability increases	ACORP main body, item C2	No
Muscimol-conjugated Magnetonanoparticles *	0.1mg/ml, $0.2x10^{-3}$ ml	Sterile saline	pressure ejection	Multiple (2-3) trials, 3-10 minutes	Labeling of sites of action of GABA agonists	ACORP main body, item C2	No

Neurobiotin	0.05 M Tris Buffer, 0.5 M KCl, pH 7.0-7.6	4-6%, .25 mL	iontophoresis (tip diameter 15-20 µL)	Once	Label neurons	ACORP main body, item C2	No
PHA-L	PBS	2%, .25 mL	Same as above	Once	Tracer (anterograde)	ACORP main body, item C2	No
CtB	PBS	1.5%,.25 mL	Same as above	Once	Tracer (retrograde)	ACORP main body, item C2	No
isoflurane	2-4%	N/A	inhalation	Once,3-4 hrs	General anesthesia for survival surgeries	ACORP appendix 5	yes
lidocaine	2%	N/A	Topical, 0.2 ml	Once, 1 minute	Inhibits gag reflex during endotracheal intubation	ACORP appendix 5	yes
xylazine	2mg/kg, 0.1ml/kg	N/A	i.m.	Once, 1 minute	Tranquilizer, aids survival surgeries	ACORP appendix 5	yes
buprenorphine	0.02 mg/kg, 0.07 ml/kg	N/A	s.c	Once, 1 minute	Analgesic, eliminates postsurgical pain	ACORP appendix 5	yes
baytril	5mg/kg, 0.1ml/kg	N/A	s.c.	Once, 1 minute	Antibiotic, prevents postsurgical infection	ACORP appendix 5	yes
atropine sulphate	0.04 mg/kg, 0.07 ml/kg	N/A	i.m.	Once, 1 minute	Antimuscarinic agent, reduce mucous secretion of trachea	ACORP appendix 5	yes
dexamethasone	0.5mg/kg, 0.25ml/kg	N/A	i.m.	Once, 1 minute	Anti-edematic agent, prevents brain edema	ACORP appendix 5	yes
saline	4ml/kg, 12ml	N/A	s.c.	Once, 1 minute	For fluid balance, prevents dehydration	ACORP appendix 5	yes

Carprofen	5 mg/kg, 0.2 ml	N/A	s.c.	Once, 1 minute	Pre-emptive and post-operative analgesia to eliminate pain	ACORP appendix 5	yes
Carbachol*	4mg/ml, $0.2x10^{-3}$ ml	Sterile saline	pressure ejection	Once, 1 minute	To induce REM sleep, for simulating naturally occurring REM sleep	ACORP main body, item C2	yes
Pentobarbital	100mg/kg, 1ml	N/A	i.v.	Once, 1 minute	For euthanasia	ACORP main body, item C2	yes
Ophthalmic ointment	N/A	N/A	topical	Once, 1 minute	For ocular lubricant	ACORP appendix 5	yes
betadine	10%, 5ml	N/A	topical	3 times, 1 minute	Used as an antiseptic prior to skin incision	ACORP appendix 5	yes
Rubbing alcohol	70%, 5ml	N/A	topical	3 times, 1 minute	Used as an antiseptic	ACORP appendix 5	Yes
Meloxicam	0.3mg/kg (0.06ml/kg)	n/a	PO/s.c.	Once, 1 minute	NSAID, post-analgesia	ACORP appendix 5	Yes
Dexmedetomidine	0.0325 mg/kg (0.065 ml/kg)	n/a	i.m.	Once, 1 minute	Sedative/analgesic, reduce heart rate	ACORP appendix 5	Yes
ketamine	6.5 mg/kg (0.065 ml/kg)	N/A	i.m.	Once, 1 minute	Short-acting anesthetic, aids endotracheal intubation	ACORP appendix 5	Yes
Butorphanol	0.65 mg/kg (0.065 ml/kg)	n/a	i.m.	Once, 1 minute	Moderate analgesia	ACORP appendix 5	Yes
Atipamezole	0.0325 mg/kg (0.0065 ml/kg)	n/a	i.m.	Once, 1 minute	Reverse the effects of dexmedetomidine, post-op	ACORP appendix 5	Yes

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm
or animals http://www.accessdata.fda.gov/scripts/animaldrugsatfda/

Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, fill out tables 2a and 2b below to explain why the use of a non-pharmaceutical grade formulation is necessary, and to describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)

Table 2a

List all items from table 2 that are not USP grade, FDA approved, a fixative, or a special diet	Why the use of a non-pharmaceutical grade formulation necessary? *Please put an X in the appropriate column, and add rows as needed.*			
	No FDA approved version exists	The FDA approved injectable forms are too dilute or have the wrong diluents for this study*	The FDA approved versions are only in pills or other forms that aren't suitable for this study	Other (please explain)
GABA	x			
bicuculline	x			
phaclofen	x			
baclofen	x			
muscimol	x			
serotonin	x			
glutamate	x			
pindolol	x			
8-OH-DPAT	x			
ACh	x			
carbachol			x	
Neurobiotin	x			
PHA-L	x			
CtB	x			

**Note: Injectables that are too concentrated can usually be diluted with saline.*

Table 2b

List all items from table 2 that are not USP grade, FDA approved, a fixative, or a special diet	How it will be ensured that the material is suitable for use? *Please put YES, N/A, or an explanation in each column, and add rows as needed.*					
	Purity/grade/pyro-genicity	Sterility	Osmolality	Stability	Formulation and pharmacokinetics	pH
	The certificate of analysis from the manufacturer will be examined to ensure the material is suitable.	If the drug does not come as a sterile solution, it will be sterile filtered before use.	Sterile USP grade isotonic diluents will be used, such as USP grade normal saline.	The supplier's guidelines on storage and stability will be followed.	The literature has been consulted to determine the appropriate formulation and that the pharmacokinetics are suitable	The pH of the solution will be tested (with pH paper or a meter) before injection
GABA	yes	yes	yes	yes	yes	yes
bicuculline	yes	yes	yes	yes	yes	yes
phaclofen	yes	yes	yes	yes	yes	yes
baclofen	yes	yes	yes	yes	yes	yes
muscimol	yes	yes	yes	yes	yes	yes

serotonin	yes	yes	yes	yes	yes	yes
pindolol	yes	yes	yes	yes	yes	yes
ACh	yes	yes	yes	yes	yes	yes
glutamate	yes	yes	yes	yes	yes	yes
8-OH-DPAT	yes	yes	yes	yes	yes	yes
carbachol	yes	yes	yes	yes	yes	yes
isoflurane	yes	yes	yes	yes	yes	yes
Neurobiotin	yes	yes	yes	yes	yes	yes
PHA-L	yes	yes	yes	yes	yes	yes
CtB	yes	yes	yes	yes	yes	yes

3. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

   a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):
   ► **For Survival surgery of implanting electrodes for chronic experiments:** cats will be pre-medicated by administering a mixture referred to as "DKT" or "Kitty Magic" (total dosage by volume is 0.2 ml/kg) which consists of a short-acting anesthetic (ketamine, i.m., 6.5 mg/kg), a sedative/analgesic (dexmedetomidine, i.m., 0.0325 mg/kg) and an analgesic (butorphanol, i.m. 0.65 mg/kg), analgesics (buprenorphine, 0.02 mg/Kg, s.c., 0.07 ml/kg; Meloxicam, 0.3 mg/kg, s.c., 5mg/ml), an antibiotic (baytril, 5 mg/kg, s.c., 0.1 ml/kg), a mucous suppressant (atropine sulphate, 0.04 mg/kg, i.m.,0.07 ml/kg), and an anti-edematic agent (dexamethasone, 0.5 mg/kg, i.m., 0.25 ml/kg).Once sedated, the glottis and the pharynx will be swapped with lidocaine (2%) to inhibit the gag reflex during endotracheal intubation. Sterile ophthalmic ointment will be applied over both eyes before shaving the hair over the surgical areas (scalp and neck). With the animal placed in a David Kopf stereotaxic head holder, the scalp and neck will be scrubbed with 3 alternating applications of betadine (10%) and alcohol (70%). Surgical procedures will then be performed under the anesthetic (isoflurane, 2-4%, via inhalation). At the completion of the surgical procedures, body-temperature warm sterile saline (25 ml/kg, s.c.) will be administered to maintain the animal's fluid balance. Post-operational care includes daily injections of Baytril (to prevent infection), Buprenorphine (12 hrs apart) and Meloxicam (every 24 hrs) as analgesics for 3 days.
   ► **For Intracardiac perfusion with fixative:** At the end of the experiment, cats will be premedicated by administering a tranquilizer (acepromazine 0.1 mg/kg, s.c.). Once sedated, the animals are euthanized using Pentobarbital (100 mg/kg, i.v.). Under deep anesthesia using pentobarbital, the thoracotomy will be performed. The animal will then be perfused with 0.9% saline. This is followed by perfusing with a fixative of 10% formaldehyde. The brain will be removed for histological studies.

   b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
   ► **For chronic (survival) cat studies:** Neurons (including NPO neurons) do not possess any pain receptors and the applied chemicals will not produce any pain sensation to the animal subjects. The various drugs will be applied by: (1) iontophoresis as ions, (2) pressure ejection in minute volumes not exceeding 0.2 microliter for each trial to adjacent NPO neurons. Therefore, no anesthesia, sedation, or tranquilization are administered for the chronic cat studies. In addition, cats will be well adapted to the

head-restraint device and the body restraining bag which will not cause any pain or distress as judged by (1) the calm behavior of the animal during recording sessions as well as (2) normal phase transitions throughout the sleep-waking cycles of the cat during experiments. Therefore, no further restraint will be necessary for the experiment. No anesthesia, sedation, or tranquilization can be used because it will interfere with the natural sleep we are studying.

Regarding the micro-application of muscimol into the DRN, we are constantly monitoring the behavior of the animal during experiments. If there was the presence of signs of pain and discomfort, such as behavioral agitation (EEG desynchrony and maximal EMG activity, failure to fall asleep), the vocalization, excessive body movements, etc, the experiment would be immediately terminated and the animal would be returned to vivarium housing. The animal will be continuously monitored for recovery until it is calm.

4. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

Name of Toxic Agent	a. Mutagen	b. Carcinogen	c. Teratogen	d. Select Agent?			e. Other – specify toxic properties (neurotoxin, etc.)
				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	
Formaldehyde	(x)	(x)	(x)	(x)	( )	( )*	( )► Acute toxicity, toxic if swallowed, severe skin burns and eye damage on contact , suspected genetic defects, may cause cancer
Bicuculline	( )	( )	( )	(x)	( )	( )*	(x)► acute oral and dermal toxicity
Phaclofen	( )	( )	( )	(x)	( )	( )*	(x)► acute oral and dermal toxicity
Muscimol	( )	( )	( )	(x)	( )	( )*	(x)► Fatal if swallowed
Muscimol-conjugated Magnetonanoparticles	( )	( )	( )	(x)	( )	( )*	(x)► Fatal if swallowed
baclofen				x			x skin and eye irritation on contact, respiratory irritation, reproductive toxicity
Serotonin				x			x toxic if swallow Acute toxicity, reproductive toxicity

pindolol				x			x harmful if swallowed; skin and eye irritation on contact
ACh				x			X skin and eye irritation on contact
Carbachol				x			X acute oral toxicity, fatal if swallow

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO▶
Date of approval▶

5. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name and BSL Number of Infectious Agent	a. ABSL Number *	b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?		
			Not a Select Agent	Select Agent used in Sub-threshold quantities	Select Agent that Requires Registration/Approval
		(Yes/No)			( )**
		(Yes/No)			( )**
		(Yes/No)			( )**
		(Yes/No)			( )**
		(Yes/No)			( )**
		(Yes/No)			( )**

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ▶
Justification for applying ABSL measures that are less protective than those recommended ▶

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO▶
Date of approval▶

6. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Biological Agent	Screening for Infectious Agents

7. **Radioactive Agents**. Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Radioactive Agent (specify the isotope)	Authorized Individual	Approving Committee or Official

8. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

Name of Agent that Contains Recombinant Nucleic Acid	Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt

9. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress

10. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk (for VA-GLA put "to be determined")
Formaldehyde	SRS	VA	to be determined
Bicuculline	SRS	VA	to be determined
Phaclofen	SRS	VA	to be determined
muscimol	SRS	VA	to be determined
baclofen	SRS	VA	to be determined
serotonin	SRS	VA	to be determined
pindolol	SRS	VA	to be determined
ACh	SRS	VA	to be determined
Carbachol	SRS	VA	to be determined
Pentobarbital	SRS	VA	to be determined
Muscimol-conjugated Magnetonanoparticles	SRS	VA	to be determined

b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.
▶The following Agent Management Plan is provided for the VMU staff and others who come into contact with these animals.

AGENT MANAGEMENT PLAN

11. Names and strains (if applicable) of agent(s), chemical(s) or toxin(s) covered in this plan: **All these drugs (listed above in table 10a) need similar PPE, etc. so we are including them in one agent management plan.**

12. ☐ Yes ☒ No: Is baseline screening of serum required for VMU animal care workers? If you are unsure if this is necessary, please contact the RBSO via email at [redacted]@va.gov

13. Discuss appropriate PPE to be worn by PI, PI's staff, and VMU staff: **Wear safety goggles for eye protection and face mask to protect inhaling chemicals in fine particle/dust and vapor form. For handling the chemicals, wear nitrile rubber gloves for skin protection. Laboratory coats are essential for whole-body protection against accidental contact with chemicals in all forms. VMU staff will not be directly exposed to any of these chemicals, since the chemicals will be microinjected into the cat's brains in minute amounts.**

14. Identify any risks associated with bedding, and precautions/handling measures to be observed by VMU staff. This includes cages/bedding returned to the VMU from work conducted in the laboratory.

There will be no risk to VMU staffs as they will not be in direct contact such as weighing, dissolution and administration of the chemicals. VMU staff will not be directly exposed to any of these chemicals, since they will be microinjected into the cat's brains in minute amounts. Most of the chemicals administered are membrane receptor agonists or antagonists and they will be converted by neurons and glia to metabolites which then will be further catabolized by the liver and renal systems to form non-hazardous final products. When alpha-chloralose is used in acute cat studies, the animal will be euthanized at the end of the experiment and the carcasses will be double-bagged in biohazard bags before disposing in the proper biohazard storage room in VMU.

15. Indicate any particular instructions/precautions regarding potential hazards for project and/or VMU staff for handling animal food and water. **The animals will receive normal food and water, no precautions needed.**

16. Discuss any special medical treatment guidelines that the project and/or VMU staff need to be aware of in case of animal medical emergency. For instance, are there specific compounds or circumstances that cannot be used or should be avoided? **All drugs are acceptable.**

17. Discuss specific health risks relative to the agent/chemical/toxin addressed in this Plan for persons who come in contact with animals/returned carcasses covered under this PSP, to include routes/mechanisms of transmission. Describe initial signs and symptoms of exposure.

With the proper personal PPE (see item 13 above), there should not be any chemical-induced health risks for people in contact with animals/returned carcasses.

In case of direct contamination of a person with the chemicals, the general route of transmission of contaminants is by skin absorption (for solid and liquid forms), swallowing, or by inhalation to the lungs (for dust forms). Once inside the body, chemicals will be transmitted to various organs via systemic (blood circulatory) system.

Common initial symptoms of chemical contaminations are skin irritation, eye damage/irritation, respiratory sensitization (coughing).

18. Discuss precautions/protocols that should be taken in case of escape of a live animal to which the agent/chemical/toxin addressed in this Plan has been administered.
Under our protocol, chemicals for experiemental studies will only be administered: (1) when the chronic cat is painlessly head-restrained, or (2) when the cat is under anesthesia (for acute study) and the head of the animal is mechanically fixed to a stereotaxic head-holder (using the mouth clamp and ear bars). Therefore, there will not be any possibility for a live animal with the administered chemicals to escape. To further prevent escape, we will keep the door of the laboratory locked at all times when the animal is in it. All researchers involved in the research protocol will be trained by taking the species-specific (cat) course (CITI program).

19. **Check all cage card requirements:**

☐ Carcinogens	☐ Infectious Agents	☒ Neurotoxins	☒ Mutagens/Teratogens
☐ Biologic Toxins	☐ Human Cells and/or Cell Lines	☐ Other (specify):	

11. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP.

[redacted]
05006-18▶
Official Date of Approval▶

ACORP Appendix 5

SURGERY
VERSION 4 V2 6-17-2015

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	Head electrodes implant			X	( )*
2	Thoracotomy for perfusion	X			( )*
3	Re-suture post-surgery (cat)		x		

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocolin addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
▶

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
▶

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

Surgery 1 ▶ Cats are prepared for chronic recordings according to procedures that are described, in detail, in Morales and Chase, 1981 (Brain Res 225:279-95), Soja et al.,1991 (J Neurosci 9:2804-11), Fung and Chase 2015 (Sleep 38:139-45). Specifically, cats will be pre-medicated by administering a mixture referred to as "DKT" or "Kitty Magic" (total dosage by volume is 0.2 ml/kg) which consists of a short-acting anesthetic (ketamine, i.m., 6.5 mg/kg), a sedative/analgesic (dexmedetomidine, i.m., 0.0325 mg/kg) and an analgesic (butorphanol, i.m. 0.65 mg/kg), analgesics (buprenorphine, 0.02 mg/Kg, s.c., 0.07 ml/kg; Meloxicam, 0.3 mg/kg, s.c., 5mg/ml), an antibiotic (baytril, 5 mg/kg, s.c,, 0.1 ml/kg), a mucous suppressant (atropine sulphate, 0.04 mg/kg, i.m.,0.07 ml/kg), and an anti-edematic agent (dexamethasone, 0.5 mg/kg, i.m., 0.25 ml/kg). Once sedated, the glottis and the pharynx will be swapped with lidocaine (2%) to inhibit the gag reflex during endotracheal intubation. Sterile ophthalmic ointment will be applied over both eyes before shaving the hair over the surgical areas (scalp and neck). With the animal placed in a David Kopf stereotaxic head holder, the scalp and neck will be scrubbed with 3 alternating applications of chlorhexidine solution and sterile water. Surgical procedures will then be performed under the anesthetic (isoflurane, 2-4%, via inhalation). Surgical drapes will be placed to cover up the areas other than the scalp and neck regions. A mid-sagittal skin incision will be made on the scalp

(from orbital to occipital bone rostrocaudally). With the skin retracted, small holes will be drilled to allow the placement of three cortical screw electrodes (bilaterally in the parietal bones; for EEG recording) and one ground screw electrode. The right frontal sinus will be exposed (for an area of 8 mm diameter) by rongeur to permit access to implant 2 screw electrodes in the orbital portion of the frontal bone for recordings of EOG of eye movement. Two flexible wire electrodes will be implanted into the nuchal musculature for recording EMG activity. A hole 4-5 mm in diameter is placed in the calvarium overlying the cerebellar cortex; it will provide for subsequent insertion of electrodes for recording or stimulating. This hole has a surrounding protective wall built with sterilized acrylic cement (chamber), and will be sealed and protected with a stainless or plastic screw closure (cap) which can be screwed on and off of a "finish" on the top of the chamber. A Winchester plug, connected to these implanted electrodes, and a chronic head-restraining device will be bonded to the skull with acrylic cement. Two nylon tubes which are instrumented to fit with the stereotaxic head restraining device will be aligned adjacent to the Winchester-connector. The connector and head restraining tubes will be bonded to the calvarium with acrylic resin of a sterile formulation. Using Ethilon monofilament nylon, the skin on the neck and around the base of the head implant will be sutured with simple, interrupted pattern. At the completion of the surgical procedures, body-temperature warmed sterile saline (25 ml/kg, s.c.) will be administered to maintain the animal's fluid balance. Triple antibiotic ointment around and inside of the hole will be topically applied. Post-operational care includes daily injections of Baytril (to prevent infection), Buprenorphine (12 hrs apart) and Meloxicam (every 24 hrs) as analgesics for 3 days. The sutures will be removed in 7 -10 days after surgery.

Surgery 2 ►Cats will be premedicated by administering a tranquilizer (acepromazine 0.1 mg/kg, s.c.). Once sedated, the animals are euthanized using Pentobarbital (100 mg/kg, i.v.). Under deep anesthesia using pentobarbital, the animal will be placed on its back and the four limbs tethered to a perfusion table. Hair over the chest will be shaved with a hair clipper. The level of anesthesia will be monitored by the corneal reflex and toe-pinch withdrawal reflex. A deep bilateral incision to the thorax will be made with a scalpel. The rib cage will then be retracted forward towards the head direction. The pericardial membrane will be cut to expose the heart. Following a cut to the right atrium(to allow the outflow of blood and perfusate), the left ventricle will be cut to enable the insertion of a perfusion guide tube to the ascending aorta. Once in position, the guide tube will then be clamped and the perfusion can begin. Blood will be rinse off the animal's body by perfusing with 0.9% saline. This is followed by perfusing with a fixative of 10% formaldehyde with 2% ferrocyanide. The brain will be removed for histology confirmation of electrode placements. The carcass will be double bagged and taken to freezer room in VMU and the perfusate will be drained into a biohazard-labeled container for proper disposal.

Surgery 3 ►► **Minor surgery to re-suture, post-surgery (cat)**: administer gas anesthesia (isoflurane) via a mask. Once the animal is anesthetized, as judged by lack of pinch/withdrawal response, clean the surgical site by using a chlorhexidine solution (alternating with sterile water 3 times). Once the site had been cleaned, administer local anesthesia (lidocaine) to ensure the animal feels no pain. Use the Germinator 500 to sterilize the Needle Holders, Scissors, and forceps before "freshening the edges of the skin" (remove the edges of the skin to make them bleed a little so that they will heal nicely) and closing the skin wound using the sterilized 5-0 Vicryl suture. The animal's heart rate and SPO2 level will be continuously monitored using the oximeter during the entire procedure.

3. **Personnel. Complete the table below for each individual who will be involved in any of the surgeries on this protocol.**

Name		Role in Surgery

	Surgery #(s) (see Item 1)	Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	1,2,3		X	X	
[redacted]	1,2 ,3	X			
[redacted]	1,2,3		x		

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
[redacted]	VMU Surgical room	1	(x)	( )*	( )*
[redacted]	VMU Room [redacted]	2	(x)	( )*	( )*
[redacted]	VMU Surgical suite	3	(x)	( )*	( )*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
▶

5. **Pre-operative protocol.**

a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

Surgery #(s) (see Item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	( ) –12hr	( ) --	(X) -- cephalic vein	( ) --
2	( ) --	( ) --	(X) -- cephalic vein	( ) --
3	( ) --	( ) --	( ) --	( ) --

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Dexmedetomidine + Ketamine +Butorphanol Aka "DKT"	1	0.0325 mg/kg, +6.5 mg/kg, +0.65 mg/kg, Total dosage by volume: 0.2 ml/kg	i.m.	once	immediate
Buprenorphine	1	0.02mg/kg, 0.07ml/kg	s.c.	2 times/day	immediate
Meloxicam	1	0.3 mg/kg, 0.06mg/ml	PO/s.c.	1 time/day	immediate
Baytril	1	2.5 mg/kg, 0.1ml/kg	s.c.	1 time/day	immediate
Atropine sulphate	1	0.04mg/kg, 0.07ml/kg	s.c.	once	immediate
Dexamethasone	1	0.5mg/kg, 0.25ml/kg	i.m.	once	immediate
Acepromazine	2	0.1 mg/kg, 0.2 ml	s.c.	once	immediate

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ►**Head implant surgery:** The animals will be pre-medicated as described above. Once sedated, the glottis and the pharynx will be swapped with lidocaine (2%) to facilitate the endotracheal intubation. Sterile ophthalmic ointment will be applied over both eyes before shaving the hair. Hair will be removed on the scalp and the neck with clippers. With the animal placed in a David Kopf stereotaxic head holder, the scalp overlying the parietal bones and the contiguous region of the neck will be scrubbed with 3 alternating applications of chlorhexidine solution and sterile water, followed by a final betadine solution spray. Surgical drapes will be placed to cover up the areas other than the zone of operation (scalp and neck regions).

Surgery 2 ►**Thoracotomy (terminal) surgery:** Cats will be given a tranquilizer (acepromazine, 0.1 mg/kg, s.c.) first, then will be euthanized using Pentobarbital (100 mg/kg, i.v.) prior to perfusion.

Surgery 3 ►► **Minor surgery to re-suture, post-surgery (cat)**: The animals will be pre-medicated as described above. General anesthesia (isoflurane) will be administered via a mask. Once the animal is anesthetized, as judged by lack of pinch/withdrawal response, clean the surgical site by using a

chlorhexidine solution (alternating with sterile water 3 times). Once the site had been cleaned, administer local anesthesia (lidocaine) to ensure the animal feels no pain.

6. **Intra-operative management.**

   a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal during surgery.

   NOTE: If saline is being administered, it must be warmed to body temperature first.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Sterile saline (0.9%)	( )*	1	4ml/kg, 12 ml	s.c. (2-3 ml per site; massage to aid in absorption)	Once (at end of surgery while the animal is still under anesthesia)
Pentobarbital	( )*	2	100 mg/kg, 1 ml	i.v.	Once
Isoflurane		1,3	2-4%	via inhalation	Continuously, vital signs will be documented every 15 minutes.

   * For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.
   ►

   b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).

   ►A water-circulating heating pad will be used to maintain the core temperature during surgery.

   Intra-operative monitoring. Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.

   ►The depth of anesthesia of the animal will be monitored using the SurgiVet Pulse-meter. Readings of the vital signs (rectal temperature, pulse rate, breathing frequency) will be recorded on a surgical log sheet at 5-minute intervals.

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery # (see Item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1	2-4 months	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)*
3	2-4 months	x						x	x

* Describe any "other" measures to be taken to maintain sterility during surgery.

▶All cortical screw and EMG wire electrodes with leads that are implanted to the animal will be autoclaved prior to implantation surgery.

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

Surgery 1 ▶Heating pad and blanket will be used to keep the animal warm.

Surgery 3 ▶ Cone collar to ensure the animal does not scratch or disrupt the new suture(s).

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1	Buprenorphine	0.02mg/kg, 0.07ml/kg	s.c.	2 times/day	3 days
1	Meloxicam	5 mg/ml, 0.3 ml/kg	P.O.	1 time/day	3 days
3	none				

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1	Baytril	2.5mg/kg, 0.1ml/kg	s.c.	1 time/day	3 days

e. Post-operative monitoring. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Continuous monitoring immediately after surgery until animal is sternal and conscious	From cessation of Isoflurane anesthesia to the point the animal is conscious and able to assume a sternal position instead of lying on its sides, vital signs will be documented every 15 minutes.	[redacted]
3	Continuous monitoring immediately after surgery until animal is sternal and conscious	From cessation of Isoflurane anesthesia to the point the animal is conscious and able to assume a sternal position instead of lying on its sides, vital signs will be documented every 15 minutes.	[redacted]

(2) Post-operative monitoring after the immediate post-operative period

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Twice daily for initial 3 days post-surgery + once daily for 10-14 days post-surgery	20-30 minutes	[redacted]

3	Once daily for 14 days post-surgery	20-30 minutes	[redacted]

f. Post-operative consequences and complications.

(1) For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1 ► Skin wounds will be swollen and sensitive. Buprenorphine and meloxicam will be administered to lessen or eliminate postsurgical pain and/or distress. Baytril will be administered to prevent infection of the animal. Softened food will be fed to the animal over the first few days postsurgery.

Surgery 3 ► Applying sutures may cause the animal to scratch. A cone collar will be worn to prevent scratching.

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1 ►Continual depression, loss of appetite, significant weight loss (over 10%), aggression, neurological signs (abnormal gait, cerebellar signs of motor deficit (e.g., ataxia and dysmetria), lethargy), abnormal phase-switching of sleep-wake cycles, failure to groom, illness refractory to veterinary intervention, wound dehiscence, and dislodgement of head implant.

Surgery 3 ► Same as listed in Surgery 1 above.

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)
►none

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	[redacted] Bldg [redacted] Room [redacted]	[redacted]	X	
3	[redacted] Bldg [redacted] Room [redacted]	[redacted]	X	

8. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

[redacted]►
05006-18►
Official Date of Approval►

ACORP APPENDIX 6

SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4 V2 6-17-2015

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
Number	Brief Description	Special Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Work	Irradiation	Imaging	Other**
1	Head and body restraint for chronic cat studies		X						
2	Electrophysiology study in sleeping cats		X						X
3	Electrical stimulation in chronic cats		X						X

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.
► We will perform intracellular recording from NPO Wake-on neurons in order to determine the changes in levels of the cellular excitability with reference to different states of consciousness (i.e., waking, NREM sleep, and REM sleep) of the animal.

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure 1 ► Following a full recovery (10-14 days) from the head implant surgery, each cat will be placed within a body restraining bag. The restraining device on the head implant will then be connected to the head pins of the David Kopf chronic cat stereotaxic head holder. The cat will be accompanied by the research staff during each period of the adaptation to the stereotaxic equipment in the lab. The cat will be restrained for an incremental, daily adaptation period from 15 min at the beginning until up to 4-5 hrs per day when full adaptation is achieved. The latter phase will be indicated by behavioral (sitting still with minimal body movements) and polysomnographic signs of normal cycling of sleep and wakefulness stages (approximately one REM sleep episode per 30 to 45 minutes during each 4-5 hrs recording session) with the head and body restrained.

Special Procedure 2 ► When the chronically instrumented animal (i.e., with head implants) becomes fully adapted to head and body restraints over a period of 2-3 weeks, daily recording sessions (4-5 hrs) will begin. We will record intracellularly NPO neurons throughout the sleep-waking cycles. This recording procedure will be performed in conjunction with monitoring the states of consciousness (waking, NREM sleep, REM sleep) based on standard polysomnographic (EEG, EOG and EMG) criteria of the restrained cat. Baseline excitability indices (e.g., membrane potential, threshold of discharging action potentials) will be recorded first, followed by changes produced by test chemicals applied. Similar control-postdrug paradigm will be used to record the cell's excitability changes during different states of waking, NREM and REM sleep.

Special Procedure 3 ► When the chronically instrumented animal (i.e., with head implants) becomes fully adapted to head and body restraints over a period of 2-3 weeks, daily recording sessions (4-5 hrs) will begin. We will record intracellularly NPO neurons throughout the sleep-waking cycles. In addition, a bipolar tungsten stimulating electrode will be employed to the DRN to deliver electrical stimulation. The purpose of the stimulation of the DRN is to antidromically activate NPO GABAergic neurons that project to the DRN by electrical stimulation of the axons or terminals of these neurons via a metal electrode consisting of a single stimulus (intensity: a few to several tens of μA; duration: 0.2 ms). In addition, the intensity of the stimulation will be slowly increased from zero to a few tens of μA to obtain a waking threshold for the stimulating site, but stimulation intensity will be kept under the waking threshold for the whole duration of experiments. For such a low intensity and short duration, and the above procedure of stimulation by gradually increasing stimulating intensity and keeping the intensity under the waking threshold, we will make every effort to minimize the pain in our animals with this particular stimulation.

b. Explain why each of these special procedures is necessary:

Special Procedure 1 ► The head restraint is necessary for successful intracellular recording of NPO neurons of the behaving cat throughout sleep and waking cycles. Accurate placement of the recording electrodes into the brain structure can only be achieved by placing the head (and the brain) in a stereotaxic head-holder (similar to human patients undergoing any stereotactic neurosurgery). Our research team is one of a handful in the world that has the unique experience and success in conducting intracellular studies in chronic cats. Our experience is that once the cat is adapted to the head restraint over a period of approximately 2 weeks, the animal subject shows no sign of distress during each experiment that lasts for 4-5 hrs. To reward the animal after each restraint conditioning session, we will play with the cat (e.g., using a laser pointer light to simulate novel moving objects around the cat), feed it with moist canned food, and watch its gait and jumping movements in the laboratory. This play-time for the cat will take half an hr before we transport the cat in a pet carrying box covered with drape back to the vivarium.

Special Procedure 2 ► The electrophysiological (intracellular recording) study is the only direct measurement of a given neuron level of activity at a given state of consciousness.

Special Procedure 3 ► The electrical stimulation is the most effective and direct way of electrophysiological studies to examine the interactions and physiological connections of different parts of brain structures by electrically exciting axons and cell bodies of neurons of a particular brain regions/structures and measuring the changes in the activity of neurons that are recorded in other brain regions/structures.

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the

individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

Procedure Number (see Item 1)	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	[redacted]	[redacted]
2	[redacted]	[redacted]
3	[redacted]	[redacted]

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number (see Item 1)	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1		Animal will show discomfort before adaptation is achieved but it will adjust to the head and body restraints and will sleep normally in the restrained condition	( )[a]	( )[b]
2	X		( )[a]	( )[b]
3	X			

a. For each procedure for which potential pain and/or distress is expected, but WILL be prevented or alleviated by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1					
2					
3					

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ▶ The cat will be restrained for an incremental, daily adaptation period from 15 min at the beginning until up to 4-5 hrs per day when full adaptation is achieved. The entire adaptation phase will take 2-3 weeks for individual cats. We will accompany the cat during each adaptation period to enable the animal to overcome its anxiety of the laboratory environment and also it will become comfortable to be around with the researcher in the room. In case the animal urinates inside the body bag, we will dry and clean up its body and abort the adaptation process for the day. Upon

full adaptation, the cat will show normal behavior of relaxed sitting when awake. It will show normal states of transitions throughout the sleep-waking cycle (approximately one REM sleep episode per 30 to 45 minutes during each 4-5 hrs recording session) with the head and body restrained. After each recording session, we will let the cat roam free within the laboratory room (with door closed and locked). The animal will have a 20-30 minutes period of play-time with pet toys, etc. We will use a laser pointer to simulate novel moving objects so the cat likes to pounce on or attempts to catch. We will reward the cat with canned food treat and water before returning it to its home cage.

Special Procedure 2 ► Same as in Special Procedure 1 above.

Special Procedure 3 ► Same as in Special Procedure 1 above. In addition, the intensity of the electrical stimulation will be slowly increased from zero to a few tens of μA to obtain a waking threshold for the stimulating site, but stimulation intensity will be kept under the waking threshold for the whole duration of experiments. Therefore, only non-painful electrical stimulation will be applied. We will be constantly monitoring the behavior of the animal during experiments. If there was the presence of signs of pain and discomfort, such as behavioral agitation (EEG desynchrony and maximal EMG activity, failure to fall asleep), the vocalization and excessive body movements, etc, the experiment would be immediately terminated and the animal would be returned to vivarium housing. The animal will be continuously monitored for recovery until it is calm.

b. For each procedure for which potential pain and/or distress is expected and <u>will NOT be prevented or alleviated</u>, provide the scientific justification for this:

Special Procedure 1 ► In case of administering tranquilizer or anti-anxiety drugs to alleviate the head and body restraint, these drugs are usually long-acting (over days). The injected drugs will then interact with the baseline recordings of NPO Wake-on neurons' activity at all states throughout the sleep-waking cycles. In order to avoid this prolonged drug effects on our intended drug-free cat study we adopt instead the restraint conditioning technique which has been proven to work with minimal or no obvious sign of distress from the head and body restrained cat. The conditioning procedure is gradual, with initially 15 min daily up to 5 hr maximal daily when fully adapted (over a period of 2-3 weeks). During the initial stage of adaptation process, we will minimize the distress of the cat by relieving the cat from the head restraint immediately when the cat shows vocalization and excessive body movements inside the restraining bag. Usually the cat is easier to adjust to be placed inside a body bag (without the head-restraint) and shows no sign of distress. The head restraint will then be re-tried the next day until the animal is adjusted to the physical restraint of the head and the body.

Special Procedure 2 ► Same as in Special Procedure 1 above.

Special Procedure 3 ► Same as in Special Procedure 1 above. In order to electrophysiologically identify NPO GABAergic neurons that project to the DRN, it is necessary to electrical stimulate the axons or terminals of these neurons in DRN. However, we will make every effort to minimize the potential pain that might be induced by the DRN stimulation. First, the stimulation will be performed as close as possible to the midline (the stereotaxic coordinates for DRN: AP 0 to -1.0, L 0, H 0 to -1.0). Second, we will apply electrical stimulation with a very low intensity and short duration. More importantly, the intensity of the stimulation will be slowly increased from zero to a few tens of μA to obtain a waking threshold for the stimulating site. Then the stimulation intensity will be kept under the waking threshold to achieve the minimal, non-painful stimulation during the whole experiment.

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number (see Item 1)	Monitoring Methods	Endpoint Criteria
1	Continual monitoring for signs of distress (vocalization, excessive body movements) and polysomnography (EEG desynchrony and maximal EMG levels, failure to fall asleep within the first 1-2 hrs being restrained).	continual depression, loss of appetite, significant weight loss (over 10%), aggression, neurological signs (abnormal gait, cerebellar signs of motor deficit (e.g., ataxia and dysmetria), lethargy), abnormal phase-switching of sleep-wake cycles, failure to groom, illness refractory to veterinary intervention, wound dehiscence, and dislodgement of head implant.
2	Same as above	Same as above
3	Same as above	Same as above

Criteria for removing animals from the head restraint device are: Presence of signs of distress (vocalization, excessive body movements) and behavioral agitation (EEG desynchrony and maximal EMG levels, failure to fall asleep within the first 1-2 hrs being restrained). If the above abnormal behavior is observed, the experiment will be immediately terminated and the animal will be returned to vivarium housing. The animal will be continuously monitored for recovery until it is calm.

Based upon our past experience, we don't expect that there will be any problem for cats to be well adapted in our recording environments with our tender care and long and slowly incremented adaptation period. As a matter of fact, we never have an incident that a cat fails to be adapted to the recording conditions. However, if a particular cat failed to the adaptation, the animal would be painlessly euthanized with the overdose of anesthesia and be perfused with fixative for conducting anatomical studies subsequently.

5. **Animal restraint.** Complete the table below for each special procedure in which animals are put under restraint for more than 15 minutes.

Prolonged Restraint (defined by the IACUC as over 15 minutes)						
Method of restraint	**Species**	**Approved Duration of Restraint**	**Method of acclimatization**	**Monitoring**	**Criteria for removing animals that do not adapt or acclimate**	**Provision of veterinary care for animals with adverse clinical consequences**

Head and body restraint (Head restrained to the David Kopf stereotaxic head-holder; body placed inside a body restraining bag)	cat	1 to 5 hrs	We adopt an incremental, daily adaptation that lasts minimally (15 min) at the beginning several days until up to 4-5 hrs per day when full adaptation is achieved. A reward strategy of play-time and canned food treat at end of each adaptation day will enable the animal to become relaxed while being restrained and accompanied by the researcher within the laboratory environment.	Continual monitoring by direct observation and polysomnography	Presence of signs of distress (vocalization, excessive body movements) and behavioral agitation (EEG desynchrony and maximal EMG levels, failure to fall asleep within the first 1-2 hrs of being restrained).	TBD by VMO

It is important to note that the head-restrained preparation is a well-established and widely accepted model used by electrophysiologists, anatomists, behavioral scientists, physiologists, etc. in the field of neuroscience research (Vanini et al., J Neurosci. 2011 31(7):2649-56; Taepavarapruk et al., J Neurophysiol. 2008, 100: 598 – 608; Levine and Jacobs, J Neurosci. 1992, 12(10): 4037-4044; May et al., J Neurosci Methods. 1991, 40: 155-169; also see our publications below). It is also a critical for us to utilize this preparation to obtain a stable and long-term intracellular recording during sleep and waking state in unanethetized, chronic cats and achieve our specific aims that are described in the grant. We have extensive experiences with this animal preparation (Drs. [redacted] and Dr. [redacted] have performed these types of experiment with survival, chronic animal preparations for more

than 30 years and more than 20 years, respectively). We have published over 45 studies using this preparation (selected peer-reviewed publication: Fung and Chase, Sleep. 2015, 38(1):139-46; Fung and Chase, J Sleep Res. 2014, 23(4):469-74; Xi and Chase, Sleep. 2010, 33(9):1236-43; Xi and Chase, Neuroscience. 2006,140(1):335-42; Xi et al., J Neurosci. 2004, 24(47):10670-8; Xi et al., J Neurophysiol. 2002, 87(6):2880-8; Xi et al., Brain Res. 2001, 901(1-2):259-64; Chase et al., J Neurosci. 1989, 9(3):743-51; Chase and Morales, Science. 1983, 221(4616):1195-8; Chase et al., Exp Neurol. 1984, 84(2):364-73; Morales and Chase, Exp Neurol. 1982, 78(2):471-6; Chase et al., Exp Neurol. 1981, 71(1):226-33; Morales and Chase, Exp Neurol. 1978, 62(3):821-7; Nakamura et al., Science. 1978, 199(4325):204-7).