ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)
Main Body
VERSION 4: (VERSION 4 MUST BE USED AS OF 1/01/14) MPLS VAHCS NOV 2013 (APRIL 2017)

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.**

   a. Contact Person: Please provide below the name and contact information for a person who can provide further information or clarification concerning this ACORP►
   [redacted]**, Brain Sciences Center** [redacted]**, Bldg.** [redacted] **Room** [redacted]**,** [redacted]

2. VA Station Name (City) and 3-Digit Station Number► **Minneapolis VA Health Care System - 618**

3. Protocol Title► **Title 1: Cellular and synaptic basis of cognitive function in prefrontal cortical networks.**

   Title 2: Characterizing thalamocortical prefrontal network dynamics underlying cognitive control in a model of schizophrenia

   Title 3: Spike Timing Defects and State Representation Impairments in Nonhuman Primates

   Title 4: Dysfunctional State Representation in Psychosis – Translating Computational Psychiatry Principles into Clinical Decision-making Indices for Patients

4. Single Animal Species covered by this ACORP► **Macaca mulatta**

5. Funding

   a. 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:
   ►(**X**) University Intramural Funds – Identify the University and Funding Component: **University of** [redacted]**, Dept. of** [redacted]**/ and Academic Investment in Research Projects (AIRP) (**[redacted]**Medical School)**
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):

   b. Funding Administrator: Indicate the entity that will be administering the research funds (check all that apply)

   ►( ) Department of Veterans Affairs.
   ►( ) MVMREF (MN Veterans Medical Research & Education Foundation)
   ►(**X**) University of [redacted]
   ►( ) Other (includes [redacted]: Specify_____

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, identify the project: (Note: this is unusual)

      (1) Title of project►

(2) If approved by the R&D Committee, give the date of approval►

b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

(1) Identify the previously approved ACORP by IACUC assigned number and title
#140702 - [redacted], Ph.D.: Title 1: Cellular and synaptic basis of cognitive function in prefrontal cortical networks. Title 2: Characterizing thalamocortical prefrontal network dynamics underlying cognitive control in a model of schizophrenia.

(2) Identify the studies described in the previously approved ACORP that have already been completed
Under the prior protocol, we trained two monkeys to perform behavioral tasks that require cognitive control, a function mediated by prefrontal cortex and disrupted in patients with schizophrenia. We have recorded neural activity in the prefrontal cortex and connected brain structures in one of these monkeys during task performance. Neural recording in the second animal and investigation of the effect of NMDA (N-methyl-D-aspartate) receptor blockade on neural activity in both animals is pending. We also initiated training of two additional monkeys to perform a category learning task. Neural recording in these two additional animals is pending.

(3) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
► 4, Macaca Mulatta. These 4 animals will be used in conjunction with 4 new animals under this renewal.

(4) 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.
► Changes made to this protocol in relation to the prior approved protocol include elimination of micro-ECoG neural recording and elimination of studies characterizing the neural mechanisms of causal inference. We no longer plan to carry out these experiments.

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(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 people 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.
► The purpose of this research is to understand how brain cells in the cerebral cortex of monkeys communicate with each other to support cognitive function, and how a breakdown of this communication between cells can lead to deficits in cognition in monkeys that are similar to cognitive deficits seen in

patients with neuropsychiatric diseases. Additionally, we want to learn whether electrical stimulation could be beneficial in improving the function of prefrontal networks to reverse these cognitive deficits. Schizophrenia impairs specific cognitive functions such as working memory and executive control. However, we have little understanding of how the disease alters the function of brain cells to produce cognitive impairment, therefore we have little basis to design more effective ways to restore cognitive function in the disease. Schizophrenia is a serious world health problem. Lifetime risk is approximately 1% for the population overall, at any one time approximately 25 million people suffer from schizophrenia. Current treatments fall short of relieving symptoms, including cognitive deficits, in most patients. This research will increase our understanding of how networks of neurons malfunction in a nonhuman primate model of the disease, information that ultimately should help identify more effective treatment approaches for patients. To learn how to effectively treat human neuropsychiatric diseases such as schizophrenia, it will be important to first understand how the disease process responsible has changed the function of neurons. This requires recording the electrical activity of individual neurons in the brain in the disease condition. Although single neuron recording in humans is in clinical trials in paralyzed patients, it is not approved for use in patients with neuropsychiatric disease. Consequently, studying altered brain function in schizophrenia at a neural level requires the development of an animal model of the human disease. This research will develop a nonhuman primate model of cortical dysfunction in schizophrenia. We will train monkeys to perform cognitive tasks that rely on the prefrontal cortex and connected brain areas (prefrontal networks), as there is ample evidence that prefrontal networks are selectively disrupted in human neuropsychiatric disease. We will introduce microelectrodes into the cerebral cortex to learn how neurons in prefrontal cortex and connected brain areas physiologically interact during normal cognitive performance. We will then administer drugs to monkeys to block the function of a synapse in the brain that is thought to be dysfunctional in schizophrenia. Specifically, we will administer drugs that block NMDA (N-methyl D-aspartate) receptors. This is a neurotransmitter receptor present at many excitatory synapses in the cerebral cortex and throughout the brain. Administering drugs which block this receptor can mimic many of the cognitive deficits found in schizophrenia in human control subjects as well as in nonhuman primates. Recent genetic linkage studies suggest that mutations that increase risk of schizophrenia and related neuropsychiatric disorders interfere with the normal operations of synapses that utilize NMDA receptors. Thus blocking these receptors with a drug is likely to mimic the malfunction of cortical circuitry that underlies human neuropsychiatric diseases such as schizophrenia. VA estimated in 2014 that as many as 120,000 Veterans receiving VA health care at that time suffered from schizophrenia (https://www.research.va.gov/topics/mental_health.cfm#research5**). Understanding how the disease disrupts the normal function of cortical networks is a first step toward devising interventions to restore function. Administration of NMDA receptor antagonists to monkeys will cause a brief period of cognitive impairment during which time they make errors on cognitive tasks that precisely mimic the errors made by patients with schizophrenia performing the same tasks. Neural recording in prefrontal networks of monkeys during the period of cognitive impairment will provide insight into how the function of neurons is changed in the disease state. We will then electrically stimulate the brain to determine whether this can restore the function of prefrontal networks and improve cognitive performance. Stimulation procedures are like those frequently used in patients, who do not report any distress or harm from the stimulation. This research will provide important new information about (1) the normal function of prefrontal networks in the support of higher cognitive function, (2) how the function of these networks is changed at a neural level when synaptic communication fails in a way relevant to schizophrenia and related diseases, and (3) whether electrical stimulation of prefrontal networks may partially reverse these changes, potentially identifying a new treatment approach for human neuropsychiatric disease. Studying brain function at the level of neurons requires introducing microelectrodes directly into the brain, which requires surgically preparing monkeys by making holes in the skull to gain the necessary access to underlying brain tissue. Similar surgical and neural recording techniques are in use to enable recording individual neurons in humans. Pain and discomfort in monkeys will be minimized in much the same way as it is in humans, by use of general anesthesia during the surgery and analgesics postoperatively. The data we obtain could provide crucial new insight into the disease process producing schizophrenia, arguably one of the worst human diseases, while at the same time identifying a new treatment approach with the potential to restore function to prefrontal networks in patients suffering with the disease.**

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.

► We will train monkeys to perform tasks that require cognitive functions thought to depend on prefrontal cortex; such as cognitive control, working memory, and categorization. Cognitive control is the ability to use rules and goals to govern cognitive processing. Working memory involves the ability to store information in an active buffer for short periods of time to manipulate that information and direct behavior. Categorization reflects the ability to recognize the similarities between objects or events that organize them into useful groups. Monkeys will view visual or hear auditory stimuli and will be required to use different rules to analyze those stimuli (executive control), store the stimuli in working memory, or assign the stimuli to categories. Monkeys will be trained to produce a motor response at the end of the trial demonstrating that the cognitive operation in question was successfully performed. The motor response will be an eye movement, hand movement or foot movement. Monkeys will be rewarded for successfully performed trials with a drop of sweetened water. As the primary goal is to understand cognitive processing in terms of the activity of individual brain cells, invasive neural recording will be necessary. To make this possible we will surgically prepare monkeys for neural recording (below), a procedure which involves, during a sterile surgery under general anesthesia, making an incision in the scalp and drilling holes (craniotomies) in the skull and implanting closable chambers over the craniotomies so that it will be possible to lower microelectrodes into the prefrontal cortex and cortical and subcortical areas that share anatomical connections with prefrontal cortex. Microelectrode recording will allow us to isolate electrical impulses in individual neurons. By recording neural activity in monkeys as they perform cognitive tasks, we can relate cognitive function to electrical activity patterns at cellular and circuit levels. By recording neural activity from more than one brain area at a time, we can characterize how neurons in prefrontal cortex synaptically communicates with neurons in other brain areas during cognitive processing. This is important because several diseases, including schizophrenia, disrupt synaptic communication between neurons in prefrontal networks. (Synaptic communication is a complex process by which electrical signals in the presynaptic cell trigger the release of a chemical neurotransmitter into the synapse, which is a specialized point of contact between neurons. The neurotransmitter then diffuses across the synapse to bind to receptor molecules on the postsynaptic neuron to modify its electrical activity.) In addition to characterizing neural activity in prefrontal networks under normative conditions, we will also modify how neurons communicate by administering drugs that interfere with synaptic function. This will partially replicate a state of synaptic dysfunction thought to cause neuropsychiatric diseases such as schizophrenia. This will allow us to reproduce in monkeys the same pattern of cognitive deficit that is observed in patients, making it possible for us to characterize the underlying defect in neuronal information processing that is responsible for those deficits. By recording from individual neurons in prefrontal cortex and connected structures at the same time during the period of cognitive impairment, we may discover how loss of synaptic communication between neurons has degraded the function of prefrontal networks. In addition, we will determine how electrical stimulation of brain areas that are anatomically connected (directly or indirectly) to prefrontal cortex modulate neural activity in prefrontal networks, both under baseline conditions and in the presence of drugs that block normal synaptic function. This will allow us to determine whether electrical stimulation of prefrontal networks can reverse the effects of synaptic dysfunction of the type thought to occur in schizophrenia and related diseases. We will stimulate prefrontal networks using both microstimulation of cortical and subcortical structures as well as deep brain stimulation targeting subcortical brain structures that are anatomically connected to prefrontal cortex.

2. **Complete description of the proposed use of animals.** 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.

► A total of 8 monkeys will be broken into 2 experimental groups (see table below). Experimental procedures are listed below in chronological order:
(Step 1) Adapting to chair restraint using positive reinforcement.
(Step 2) Initiating and maintaining the scheduled fluid access protocol.
(Step 3) Operant conditioning (using positive reinforcement) to perform cognitive tasks that involve analyzing visual and/or auditory stimuli and making eye, hand or foot movements.
(Step 4) Structural Magnetic Resonance (MRI) and computed tomography (CT) Imaging.
(Step 5) Test effects of NMDAR antagonists ketamine and/or phencyclidine (PCP) on cognitive

performance.
(Step 6) Survival surgery #1 to implant mechanical devices for head restraint onto the skull. (Step 7) Recovery from surgery #1.
(Step 8) Training with head restraint.
(Step 9) Survival surgery #2 to prepare monkeys for neural recording and electrical stimulation. The minimum time between surgery #1 and #2 will be 4 weeks.
(Step 10) Recovery from surgery #2.
(Step 11) Record neural activity in prefrontal cortex and/or connected brain areas during baseline conditions and following administration of NMDAR antagonists.
(Step 12) Position deep brain stimulation (DBS) electrodes in the target subcortical brain areas.
(Step 13) Obtain CT scan to localize subcortical neural recording and DBS stimulation sites.
(Step 14) Test the effects of neural stimulation on neural activity and cognitive performance under baseline conditions and following administration of NMDAR antagonists.
(Step 15) Application of antimitotic agents to slow thickening of the dura within the microelectrode recording chambers.
(Step 16) Periodic scraping of the dura mater to reduce its thickness within the microelectrode recording chambers.
(Step 17) Survival surgery #3 to prepare monkeys for neural recording and electrical stimulation in 2nd brain hemisphere. The minimum time between surgery #2 and #3 will be 4 weeks.
(Step 18) Recovery from surgery #3.
(Repeat of Steps 12 – 17 above with neural recording and electrical stimulation in the 2nd cerebral hemisphere).

If possible, the survival surgeries outlined above will be reduced to 2 or 1 instead of 3. For example, it may prove possible to combine implantation of head restraint devices onto the skull and preparing the monkeys for neural recording and stimulation by making holes in the skull and implanting plastic chambers over the holes into a single surgery. This can be an issue because once holes in the skull are made, the dura mater within the hole covering the brain starts to thicken. If a protracted period of additional training using head restraint is necessary, the dura may become too thick to allow electrodes to readily penetrate the dura to record neural activity in the brain; limiting the amount of neural data we would be able to collect. This would warrant implanting head restraint devices, training with head restraint to achieve stable performance, and then making the holes in the skull afterward in a separate surgery (to minimize the time the dura was exposed before neural recording commenced). However, if possible, implantation of head restraint devices and preparing the monkeys for neural recording and stimulation in the 1st brain hemisphere will be collapsed into a single surgery. The maximum number of craniotomies and chambers that a monkey will receive is 4.
The brain areas in addition to prefrontal cortex where neural recording and electrical stimulation will be conducted may include other cortical areas that are connected to the prefrontal cortex (such as the cingulate, parietal, premotor, superior temporal or entorhinal cortex, or the hippocampus), or other subcortical areas that are connected to the prefrontal cortex (such as the mediodorsal nucleus of the thalamus, the pulvinar, or the caudate putamen).

Experi-mental Group	Number of monkeys	Experimental manipulations	Behavioral tasks	Experimental objectives
1	4	Neural recording and electrical stimulation in prefrontal networks, with and without concurrent NMDA receptor antagonist administration to characterize the role of NMDA receptors in neural mechanisms of cognitive control and	Working memory, cognitive control. These cognitive processes are disrupted in schizophrenia.	Characterize the change in neural and network activity caused by loss of NMDA receptor function. Identify the changes in neural function that underlie errors in performance of monkeys like the errors committed by patients with schizophrenia performing the same cognitive tasks

		working memory		
2	**4**	**Neural recording and electrical stimulation in prefrontal networks, with and without NMDA receptor antagonist administration to investigate role of NMDA receptors in category learning**	**Category learning. These experiments will record neural activity as monkeys learn new sets of categories through trial-and-error feedback during the period of neural recording. This will enable us to relate changes in cognitive performance to changes in neural activity and the strength of neuronal interactions during learning**	**Extend the investigation of the role of NMDA receptors to neural and behavioral correlates of learning. Category learning is likely to depend on prefrontal networks and NMDA synaptic mechanisms that (1) are disrupted in schizophrenia, and (2) mediate experience dependent synaptic plasticity. By characterizing how changes in cognitive function and neural activity during learning depend on NMDA receptors, these studies will inform future studies investigating disrupted learning and neural plasticity in schizophrenia.**

Each animal will participate in the study until: (a) we have obtained sufficient neural data (defined below), (b) the acquisition of additional neural data has become impractical (due primarily to the rate of electrode breakage), or (c) the health of the animal is compromised to the point that euthanasia is indicated (see T. Endpoint Criteria). We anticipate acquiring neurophysiological data from between approximately 200-400 neurons per recording chamber, per drug condition and per monkey. The exact number of neurons recorded in each chamber is a function of a combination of factors: including variability in the accuracy of surgical targeting of brain structures of interest, variability in the behavioral performance of the monkeys, variability in the condition of the dura and frequency of electrode breakage, and the health of the monkey, among other factors. In practice, once 200-400 neurons have been recorded per brain area studied and per drug condition, for a total of 400-800 neurons per chamber, the animal is sacrificed.

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

► Four monkeys are required in Groups 1 and 2 because whereas using 2 monkeys per study remains the standard in the field for publication of neurophysiological results using monkeys, we have found that the physiological response of prefrontal networks to pharmacological manipulation of synaptic function can differ between individual animals. Recording from 4 monkeys per group will serve to mitigate this variability, and provide better estimates of the effects of NMDA receptor blockade on neural network activity that are likely to generalize across animals. However, our units of statistical analysis to test hypotheses will be based on the number of neurons rather than the number of individual animals. We anticipate obtaining neurophysiological data from 200 – 400 neurons per brain area per drug condition per monkey. Recording this number of neurons has proven sufficient to detect significant relationships between neural activity and behavioral performance as well as significant changes in neural activity resulting from pharmacological blockade of NMDA receptors in our prior studies under the preceding ACORP.

This proposal incorporates experiments that seek to relate changes in synaptic function to changes in neural function and behavior in two sets of behavioral tasks. The first set will investigate neural mechanisms of working memory and executive control. Deficits in working memory and executive control have been extensively documented in schizophrenia, and in the first set of experiments we will translate established behavioral paradigms developed to study these cognitive deficits in patients to monkeys. In the second set of experiments, we will investigate neural mechanisms of category learning. Category learning is a form of learning that is likely to depend on prefrontal networks and NMDA receptor mechanisms that are disrupted in schizophrenia. In these experiments, monkeys will assign visual

stimuli to categories per a flexible rule they must discover based on trial-and-error feedback within a block of trials. As they learn new categories, the neural signals and interactions that mediate categorization will emerge de novo in prefrontal networks. This will allow us to relate cognitive flexibility to neural plasticity as the brain learns. These experiments will increase our understanding of the neural mechanisms of learning in prefrontal networks as well as the dependence of these mechanisms on NMDA synaptic mechanisms. The data should provide information to inform future studies to characterize how neural and behavioral plasticity mechanisms are disrupted in schizophrenia.

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.)
► **The following procedures will be performed in chronological order.**

(Step 1) Adapting to chair restraint using positive reinforcement. Monkeys will be trained to move from their home cages to a primate chair using the pole and collar restraint system coupled with positive food reinforcement and clicker training. The primate chair (on a wheeled cart) will be placed inside a mobile enclosure, which will then be wheeled to the laboratory. In the laboratory, monkeys will be adapted to increasing periods of chair restraint using positive food reinforcement. Initially, chair restraint will last for approximate 10 – 20 minutes, but be gradually extended to several hours. As monkeys adapt to chair restraint, they typically sit quietly throughout.

(Step 2) Initiating and maintaining the scheduled fluid access protocol. To train monkeys to perform behavioral tasks, it is necessary to schedule their access to fluid, and deliver a fluid reward for each successfully performed trial. This provides the motivation that makes training possible. During behavioral training sessions, the fluid intake of the animals is carefully controlled. The animals get a substantial amount of water during the training sessions and this amount is supplemented to a minimum daily amount of 20ml/kg, if not enough fluid was consumed during behavioral training or testing in each day. During periods of the scheduled fluid procedure, the condition of the animals for possible dehydration is tested by measuring the urine specific gravity periodically (as frequently as practical depending on the availability of fresh uncontaminated urine specimens in the pan underneath the home cage) and body weight at least once a week. Urine specific gravity measurements will also be taken several (~3) times prior to the onset of scheduled fluid procedure to provide a baseline level for the individual animal. Hydration will be adjusted to maintain urine specific gravity at a reading of approximately 1.040 or less. Moreover, during training periods the animal is given water ad libitum overnight every 7 days, with the following exception*.

*Some animals develop the habit of "tanking up" during this overnight ad libitum, and can carry this higher hydration level over into the next few days. This elevated hydration level reduces the animals' motivation to perform the task in which they have been trained; in general, this occurs during the first two days of the week thereby significantly prolonging the duration of the experiment. Therefore, to achieve a more regular, stable performance in such animals, and after consultation with the veterinarian and documentation in the animal's medical record, we will reduce the overnight ad libitum to an amount approximately 1.25 – 3 times the minimum daily intake (25-60 mL/Kg.): about 200 – 600 mL ('restricted ad libitum'). The absolute minimum for day 7 during this restricted ad libitum period would be 25 mL/Kg. In these special cases, we will be especially diligent in monitoring the animals' hydration level through more frequent use of Urine Specific Gravity (USG) measurements; during the first week of implementation, attempts will be made to take this measurement daily and two times each week thereafter until the end of the restricted ad libitum period. We will weigh the animals several times each week and monitor their food intake. Changes in appearance and behavior will also be considered in determining hydration status. The monitoring of hydration is complex and is based on multiple factors, not urine specific gravity alone. During this period, the veterinarian will be kept informed of any deviation of the various measures from the normal values. Restricted ad libitum would be limited to three weeks out of every four; e.g. at least one day of true ad libitum water would occur per month.
When the animals are not in training, water is provided ad libitum.

(Step 3) Operant conditioning (using positive reinforcement) to perform cognitive tasks that involve analyzing visual and/or auditory stimuli and making eye, hand or foot movements. We will monitor monkey's eye position noninvasively using a video infrared eye tracking system. Monkeys will receive a drop of liquid reward for each correctly performed trial of various cognitive behavioral paradigms (~ 0.1 – 0.3 ml; Tang or

similar). First monkeys will be trained to direct their gaze toward visual targets on a video monitor for liquid reward. Then they will be trained to maintain fixation of the gaze target while other visual stimuli are presented at various locations in the display for liquid reward. In addition, we may present auditory tones to provide success/failure feedback to the monkey, or in combination with visual stimuli. Then monkeys will be trained to produce simple eye, hand, or foot movements in response to the stimuli presented, for liquid reward, based on increasingly complex rules. The cognitive tasks we will use will require cognitive functions thought to rely on prefrontal cortex such as working memory, cognitive control, and categorization. Motor responses will consist of saccadic eye movements, hand movements to move a joystick or depress a lever, or foot movements to depress a lever. Each trial will last approximately 5 – 30s in total duration, and trials will be separated by an intertrial period of one to several seconds. Once fully trained and adapted to the task, monkeys typically work for between 600 and 1000 trials during each daily training session.

(Step 4) Structural magnetic resonance imaging (MRI) and computed tomography (CT) imaging. To accurately localize brain regions for neural recording, we will obtain structural MRI and CT scans (on different days) of each monkey prior to surgical placement of craniotomies, recording chambers. Monkeys will be given ad libitum access to water, and fasted, for 12-24 hours before the scan. On the day of either the MRI or CT scan, we will first transport the monkey from the housing facility to the laboratory, and then administer an IM injection of ketamine (7.0 mg / kg) and xylazine (0.6 mg / kg) to initiate anesthesia (Please see Appendix 6 for additional details). Once sufficient anesthesia is achieved we will position the head of the monkey in an MRI/CT compatible stereotaxic frame, and then place the monkey and the stereotaxic frame on a gurney. We will then cover the monkey and frame with drapes, and transport the monkey from [redacted] to the MRI or CT facility in [redacted]. (Please see Appendix 7 for additional detail regarding Use of Patient Care Equipment and/or Areas). [redacted] Monkeys are placed in the scanner and structural MRI images acquired. Once the scans are complete, the monkeys are removed from the stereotaxic frame, and then returned first to the laboratory, and then to their home cage.

(Step 5) Testing effects of the NMDAR antagonists ketamine and/or phencyclidine on cognitive performance. Monkeys will perform cognitive tasks during chair restraint in the behavioral testing room within the laboratory. We will collect behavioral performance for a baseline period. We will then administer an IM injection of an NMDA receptor antagonist, either ketamine or phencyclidine (PCP), in the quadriceps or deltoid muscle. Monkeys will receive at most one injection of an NMDA receptor antagonist per day of behavioral testing. We will administer ketamine (Ketaset solution; concentration 100 mg/ml) within the dose range of 0.05 – 4 mg/kg IM. This dose range has been previously used in nonhuman primates without adverse effect (Taffe et al., 2002; Condy et al., 2005; Stoet and Snyder, 2006; Buccafusco and Terry, 2009; Castner et al., 2010; Blackman et al., 2013). Alternatively, we will administer PCP within the dose range of 0.15-0.3 mg/kg PCP. PCP is a longer lasting NMDA receptor antagonist relative to ketamine and can have adverse effects (see below). However, we do not anticipate adverse effects from PCP in this dose range. We will closely monitor monkeys for a period of approximately ¼ - ½ hour to make sure that no idiosyncratic or adverse reactions to PCP occur before initiating behavioral testing. After injecting NMDA antagonists (either ketamine or PCP), we will collect additional behavioral data during task performance to characterize cognitive deficits in task performance produced by NMDA receptor blockade.

(Step 6) Survival surgery #1 to implant mechanical devices for head restraint onto the skull. For a detailed description of surgical procedures, please see Appendix 5. All surgeries will be sterile and performed under gas anesthesia (1-4% Isoflurane). The first surgery will be performed to implant biocompatible devices (posts or blocks) on the skull to allow us to stabilize head position. In brief, monkeys will be fasted for 24 hours, and be given access to water ad libitum for 24 hours or more, prior to surgery. On the day of surgery, monkeys will be anesthetized with an injection of ketamine (7.0 mg/kg) and xylazine (0.6 mg/kg), we will shave the scalp, place an intravenous catheter in the saphenous vein in the back of the calf, start an IV sterile saline drip, place the monkey on a surgical table, introduce an endotracheal tube, and initiate gas anesthesia. Once sufficient surgical anesthesia is induced (as judged by absence of pinch or blink reflexes), we will place the monkey in a stereotaxic frame, disinfect the scalp, cover the animal in sterile surgical drapes leaving an aperture over the scalp, make either a single midline incision in the scalp, or two incisions in a cross pattern, retract the scalp, drill small holes in the skull, place screws through feet on posts or blocks of biocompatible material, and screw them into the holes made in the skull. We will surround the implanted pieces with either dental acrylic or bone cement, and suture the skin such that the wound margin closely conforms to the implanted pieces. We will administer injectable

analgesics (see Appendix 5), remove the monkey from the stereotaxic frame, terminate gas anesthesia, remove the endotracheal tube, allow the monkey to recover to the point of being able to maintain body posture, place the monkey in a primate chair, and recover them further in the lab maintaining body temperature with water circulating heating pads, warm blanket wraps and heat lamp. During the recovery time, if the duration of sedation during recovery from anesthesia permits, we will fix metal tabs to the threaded tops of the implanted posts and then screw the tabs to a halo head holding device. During subsequent training, we will bolt the halo to an external post for head stabilization. Once monkeys acquire a normal level of motor function and alertness, we will return them to their home cage.

(Step 7) Recovery from surgery #1. Monkeys will be allowed to recover with ad libitum access to food and water in their home cage for a minimum postsurgical recovery period of 3-5 days, or a longer period if required so that their behavior appears normal, they are eating and drinking normally, and moving normally. If it was not possible to attach the halo to the posts during the initial surgical recovery period (because recovery from anesthesia was too rapid), after a minimum of two days following surgery #1, we will chair the monkey and administer ketamine (2-10 mgs/kg) in the recording lab to sedate them for a brief period while we attach the halo to the posts.

(Step 8) Training with head stabilization. The halo attached to the posts will be screwed to an external brace attached to the primate chair to stabilize head position. Monkeys will be given food treats when the head is stabilized. Then monkeys will be placed in the behavioral testing chamber, behavioral testing will commence, and monkeys will work for liquid reward. Monkeys will be continuously monitored while they adapt to head fixation. Training with head fixation each day will continue if monkeys remain calm and relatively still during behavioral performance. Should they start to struggle consistently, they will be removed from the behavioral testing apparatus and their head released. Monkeys readily adapt to head fixation, and the period over which they are willing to sit quietly without struggling typically increases rapidly from a period of about 20 minutes per day to a period of several hours per day.

(Step 9) Survival surgery #2. Please see Appendix 5 for additional detail regarding surgical procedures. Following implantation of the head posts, the cranial implant will consist of several posts each surrounded by a small amount of bone cement or dental acrylic protruding through the scalp, which will have been sutured close around the posts. Monkeys will be anesthetized with ketamine/xylazine, an IV catheter will be placed, a saline drip initiated, the monkeys will be intubated, placed on gas anesthesia, put in a stereotaxic frame, the monkeys draped, and the surgical field will be disinfected as described in Step 6 above. The scalp in between the posts will be cut to expose the skull over target brain areas for neural recording. The center of the craniotomies will be localized on the skull using a micromanipulator arm attached to the stereotaxic frame. We will make 1-3 craniotomies in surgery #2. This will allow us to record and electrically stimulate within prefrontal cortex (craniotomy 1) and up to two connected brain structures. Connected brain structures could include areas of cortex (such as the parietal or cingulate cortex) or subcortical structures (such as the thalamus or basal ganglia) that share anatomical connections with prefrontal cortex. We will obtain MRI and CT images of each monkey prior to surgery, import the images to Cicerone (a 3D image reconstruction application) and position to-scale 3D renditions of the neural recording chambers over their cortical and/or subcortical targets. This will allow us to position recording chambers within space constraints imposed by the available surface area of the skull. We will make craniotomies either using a trephine or a hand held high-speed drill. We will remove the bone flap exposing the dura mater covering the cerebral cortex. We will use a high-speed drill to make small holes surrounding the craniotomies and place screws in these holes. In the case of microelectrode recording in a brain area, we will place plastic recording chambers with tight-fitting caps over the craniotomies, and cement the chambers to the screws in the skull using bone cement or dental acrylic.

(Step 10) Recovery from surgery #2. Monkeys will be allowed to recover with ad libitum access to food and water in their home cage for a minimum postsurgical recovery period of 3-5 days, or a longer period if required so that their behavior appears normal, they are eating and drinking normally, and moving normally.

(Step 11) Record neural activity in prefrontal cortex and/or connected brain areas in 1st brain hemisphere during baseline conditions and/or following administration of NMDAR antagonists. Monkeys will be brought to the lab in a primate chair. We will stabilize head position, open the recording chamber(s), and rinse the chambers with saline. We will move the monkey into the recording enclosure, lower electrodes into the recording chamber, isolate the activity of individual neurons in the cortex and/or subcortical brain area, and record

neuronal activity while the monkey performs a baseline set of trials of the cognitive task(s), for a period of approximately 30 mins (see below). We will then suspend neural recording, and inject the monkey in either the deltoid or quadriceps muscle with an NMDAR antagonist (either phencyclidine or ketamine), or an equivalent volume of sterile saline (as control). In the case of phencyclidine injections, we will inject at a dose of 0.15-0.3 mg/kg IM. In the case of ketamine injections, we will inject at a dose of 0.05 – 4 mg/kg IM. Each antagonist has specific advantages. We will use PCP as our first choice for NMDAR antagonist to test as our prior experiments have shown that monkeys tolerate it well and it produces a longer lasting period of cognitive impairment relative to ketamine. This allows recording neural activity for a longer period of time while the cognitive deficit is still present, which provides a better estimate of how neural activity has changed in response to the drug. Ketamine is shorter acting and this also has advantages. Its shorter time of action may make it possible to record neural activity during washout, for example, to observe neural firing patterns return to normal. This helps establish that changes in neural activity are due to acute NMDAR blockade rather than some more longer lasting effect. We have used Ketamine as an anesthetic and test substance, and have not noted tolerance to develop to the drug that would limit the ability to obtain a reliable and repeatable cognitive deficit within the dose range specified above. Recording neural activity during washout for PCP would not be practical typically as cognitive effects last for several hours and it is not routinely practical to maintain stable recordings of neurons for this extended period. Following the injection, we will recommence neural recording. Alternatively, we will inject monkeys IM with either NMDAR antagonists or saline with the head freely moving (before we fix head position at the start of neural recording). We will then slowly advance microelectrodes through the dura and into the prefrontal cortex and connected brain areas. Each monkey will serve as its own control in both Groups 1 and 2. The experimental condition will consist of neuronal activity patterns recorded after injection of NMDAR antagonists. The control condition will consist of neuronal activity patterns recorded after injection of saline. In addition, we will collect neural data for several days before initiating NMDAR antagonist injection to characterize baseline activity patterns before exposure to NMDAR antagonist. In the case that we inject NMDAR antagonist or saline in the deltoid or quadriceps muscle after isolating neuronal activity, each group of neurons will serve as its on control in addition and we will be able to characterize activity patterns in the same group of neurons before and after NMDAR antagonist administration. We will alternate injections of saline and NMDAR antagonist on a daily basis (one injection of either NMDAR antagonist or saline per day). One day after injection of NMDAR antagonist is sufficient for behavioral performance to return to normal, indicating this is a sufficient wash-out period to recover normative cortical network function that underlies successful behavioral performance. We will use microelectrode drives containing 16 independently movable glass coated microelectrodes, or microelectrode drives advancing linear electrode arrays with multiple recording sites along a single shaft. The microelectrodes are robust enough to penetrate the dura (while the dura remains relatively thin) without a guide tube, and the microelectrode drive is designed to be used without guide tubes. However, the linear electrode arrays are fragile, will not penetrate the dura on their own, and therefore a guide tube is required. The microdrive we will use with the linear electrode arrays is designed to be used with guide tubes. Therefore, when using the linear electrode arrays, we will use beveled guide tubes to puncture the dura, and then slowly advance each linear electrode array through a guide tube and into the brain. Once the microelectrodes and/or linear electrode arrays are positioned within target brain structures, we will record neural activity during task performance. Daily recording will continue until we have recorded the activity of approximately 200-400 neurons per brain area per monkey, and in the case of Group 1, per drug condition (saline or NMDAR antagonist). We can typically record about 25 neurons per brain area per day, so this requires approximately 8 – 20 injections of NMDAR antagonist per monkey per hemisphere of neural recording. The neural recording procedures are painless, and monkeys sit quietly throughout. We will adjust the depth of the microelectrodes until most of them pick up the electrical impulses of individual neurons, then record neural activity during task performance.

Acquisition of NMDAR antagonists. Ketamine and phencyclidine are U.S. Drug Enforcement Administration (DEA) controlled substances (Schedule III and Schedule II, respectively). Therefore, they will be purchased through the VA Pharmacy by personnel with the appropriate DEA licenses to purchase controlled substances.

Potential adverse effects to NMDA receptor antagonists. PCP, like ketamine, is an NMDA receptor antagonist and an anesthetic, and monkeys are primarily sedated after administration of the drug. The advantage of PCP over ketamine is that it is longer acting, produces a longer period of cognitive impairment, making it possible to record more neural activity. PCP can induce cognitive deficits and clinical symptoms in human control subjects which are very like those seen in patients with schizophrenia. PCP intoxication in humans can produce brief periods of irregular breathing (apnea and tachypnea), and muscular rigidity (dystonia). However respiratory depression requiring intubation following PCP intoxication in humans is uncommon (Bey and Patel, 2007). We have observed brief periods of irregular breathing and muscular rigidity in monkeys for a period of several minutes

immediately following injections of PCP. These effects resolved spontaneously without intervention and monkeys recovered fully without adverse effects. In humans, PCP intoxication or overdose can also induce cardiac arrhythmias, and rhabdomyolysis, which is break down of muscle tissue that adversely affects kidney function if severe (Bey and Patel, 2007). These adverse effects are not anticipated at the doses of PCP we will employ. We observed seizures in one monkey given ketamine during maintenance of a cranial implant, which had previously undergone PCP administration in conjunction with neural recording. In consultation with a laboratory animal veterinarian, we treated with dexamethasone and diazepam, and the seizure resolved without lasting impairment and did not recur. Adverse behavioral reactions to PCP including exaggerated motor reactions to acute stressors have been reported after chronic NMDA receptor blockade at doses and for durations more than those proposed here (Linn et al., 1999). Immediately following injection of PCP, and for several hours afterwards, monkeys will be closely monitored. If any adverse reactions such as those described occur, we will immediately consult with a laboratory animal veterinarian to determine whether to administer diazepam (0.5 – 1.0 mg/kg IV, IM, or rectally), which can effectively mitigate many of these reactions. If breathing is irregular for a protracted period and assistance with breathing is necessary, we will artificially ventilate the animal with a pediatric Ambu bag resuscitator until spontaneous respiration is achieved. We will then consult with the veterinarian afterwards to determine whether to lower the dose of NMDA receptor antagonist, switch to the less potent NMDA receptor antagonist ketamine, or terminate drug treatment altogether. PCP administration is warranted because this drug produces the best pharmacological model of altered cognitive function and cortical information processing in schizophrenia (Kantrowitz and Javitt, 2010). Therefore, administration of the drug provides the best means available to temporarily mimic the neural dysfunction producing this serious human disease in nonhuman primates. In our experience, the drug can be safely administered to nonhuman primates at the doses specified in conjunction with neural recording.

(Step 12) Position deep brain stimulation (DBS) electrodes in the target subcortical brain areas. To test the effects of electrical stimulation on neural activity and cognitive performance, small electrical currents will be delivered into targeted neural structures either through microelectrodes (the same electrodes used to record neural activity) or deep brain stimulation electrodes designed to deliver electrical current to deeper structures (such as the thalamus or basal ganglia). In the case that we utilize deep brain stimulation probes, the procedures for probe implantation are done in an awake monkey. Techniques like this are performed in awake, behaving human patients with no harm or distress reported. To minimize any potential discomfort associated with insertion of the DBS electrode, we will apply 1-3 ml of Lidocaine HCL (1%) to the surface of the dura inside the chamber, wait ~3 minutes, and then remove excess lidocaine with cotton swabs. After we have performed neural recording to map the subcortical target (Step 11), we will then insert a sterilized stainless steel guide tube through the dura into the brain using a Microdrive until the lower end of the guide tube is situated ~5-10 mm above the subcortical target brain area. We will then insert a sterile deep brain stimulation (DBS) electrode through the guide tube so that the tip of the DBS electrode protrudes from the end of the guide tube ~5-10 mm. At this depth, the contacts for neural stimulation on the DBS electrode will be located within the target subcortical structure. We will then remove the guide tube, and cement the tip of the DBS electrode to a support inside the recording chamber to hold the DBS electrode permanently in place, and then close the recording chamber. We will then attach the wire leads of the DBS electrode to a connector, and attach the connector to the cranial implant. Once positioned, we will leave DBS probes implanted chronically to allow repeatable stimulation of the same neural structures (mimicking the chronic implantation of probes currently in clinical use in human patients to treat Parkinsonian tremor). Chronic implantation of DBS probes through neural recording chambers similar to those we will implant has been successfully maintained in nonhuman primates for extended periods without adverse effect by colleagues at the University of [redacted] (Agnesi et al., 2013; Johnson et al., 2009), using standard chamber maintenance techniques. The DBS probes will by necessity pass through overlying cortical areas and subcortical white matter tracts in route to the target subcortical brain area. The probes themselves are thin (outer diameter, 0.7 mm), and so we anticipate that neural damage caused by the probe will be minimal. However, should motor symptoms (such as limb weakness) or other adverse reactions emerge after DBS probe implantation, we will consult with a veterinarian to determine whether to remove the probe, and either try another site (if symptoms resolve), or terminate DBS stimulation.

(Step 13; Group 1) Obtain CT scan to localize subcortical neural recording and DBS stimulation sites. We will check the placement of the linear electrode arrays as well as DBS probes through the acquisition of a CT scan (scan procedures including how the monkeys will be anesthetized, placed in a stereotaxic frame, and scanned, will be as described for MRI scan acquisition in Step 4, above). We will obtain CT scans with the linear

electrode arrays or DBS probe in place in the brain so their location can be imaged. The position of the DBS probe within the recording chamber will be moved using aseptic technique if it is not in the target area as seen with the CT scan or we are not observing any changes in cognitive performance and/or neural activity during neural stimulation, indicating that the probe is not in a good location for affecting prefrontal networks. We will at most attempt 3 different DBS probe positions. Once the scans are complete, the monkeys are removed from the stereotaxic frame, and then returned first to the laboratory, and then to their home cage.

(Step 14) Test the effects of neural stimulation on neural activity and cognitive performance under baseline conditions and following administration of NMDAR antagonists. We will evaluate the potential of neural stimulation as a therapeutic approach to counteract the damaging effects of reduced synaptic function on neural activity patterns and cognitive function in a primate model of human neuropsychiatric disease. Neural stimulation will be performed by passing small electrical currents through either the microelectrodes or the DBS electrode to activate surrounding neural tissue. Neural stimulation will be performed during both baseline conditions (e.g. without drug or after injections of saline) and following injection of NMDAR antagonists, as described above. *DBS stimulation procedures.* Deep brain stimulation will be administered with a human DBS probe that has been scaled down for monkeys. We will deliver continuous monopolar or bipolar stimulation consisting of biphasic current pulses between 30 and 100 μs in width at frequencies between 30 and 175 Hz, and amplitudes between 50 μA – 1.5 mA. Comparable deep brain stimulation parameters have been utilized without adverse effects in nonhuman primates previously (Agnesi et al., 2013; Johnson et al., 2009), including in the primate mediodorsal nucleus of the thalamus (Smith et al., 2009). We do not anticipate that DBS will induce seizures as a side effect, because seizures have not been reported in prior studies using these parameters in monkeys (Agnesi et al., 2013; Johnson et al., 2009; Smith et al., 2009), and seizures are a rare side effect of DBS in humans, observed in less than 0.5% of cases (Coley et al., 2009). *Microstimulation procedures.* To activate small regions of neural tissue surrounding the tips of microelectrodes, we will deliver biphasic current pulses at frequencies from 1 – 300 Hz, and amplitudes from 5 to 300 μA. Pulse width will be 1 – 500 μs. Microstimulation is a standard neurophysiological technique in nonhuman primates and no adverse effects are anticipated. Similar microstimulation parameters have been employed in nonhuman primates previously without adverse effect (Histed and Miller, 2006).

(Step 15) Application of antimitotic agents to slow thickening of the dura within the microelectrode recording chambers. The microelectrodes we use to record neural activity are robust enough to penetrate the dura during the period that it is relatively thin. However, the dura within the recording chambers thickens progressively with time after the craniotomies are made to a point where the microelectrodes break before entering the cerebral cortex. Since it is not feasible to use guide tubes with the microelectrode system, it will be important to slow the thickening of the dura and lengthen the period of successful neural recording using microelectrodes. For that purpose, we may briefly apply a 2.5% solution of 5-fluorouracil, an antimitotic agent, on the dura at the end of each recording day for a period of 5-10 minutes and then wash it off with saline, before closing the recording chamber and returning the animal to its home cage. The 2.5% solution is used for topical treatment of skin lesions that are potential precursors of carcinoma. Other groups have used an undiluted solution successfully to extend the period of transdural neurophysiological recording in nonhuman primates (Spinks et al., 2003). If an antimitotic agent is needed, we will use the 2.5% diluted solution as it is less potent and easier to handle.

(Step 16) Periodic scraping of the dura mater to reduce its thickness within the microelectrode recording chambers. Application of 5-fluorouracil (if used) will slow but not prevent thickening of the dura. Therefore, it will become necessary to scrape the dura mater within recording chambers periodically during neural recording. Once the dura has thickened to a point where microelectrodes routinely break, we will scrape the dura not more than twice a week to allow us to continue to record neural activity. For the scraping procedure, we will use surgical tools to partially dissect superficial layers of the dura. We will perform the procedure in a neurophysiological recording laboratory on the second floor of [redacted]. To perform this procedure, we will sedate monkeys by administering ketamine (2-10 mgs/kg), or if a longer period of sedation is required, a mixture of ketamine (7.0 mg / kg) and xylazine (0.6 mg / kg). If it is necessary to extend the period available to complete the procedure, we will redose the monkey once with one half the dose of ketamine initially administered. To mitigate any pain associated with dural scraping, we will administer Meloxicam (0.2mg/kg SQ first day, 0.1mg/kg subsequently) once a day, starting the day of the dural scraping procedure, for a minimum of 1 day, or for a period necessary to mitigate any behavioral signs of continuing pain, such as lethargy or reduced appetite, as

determined by Staff and in consultation with a Staff Veterinarian. In the rare event that the scraping the dura produces a tear in the dura, we will cover the dura with gelfoam or gelfilm, and close the chamber. Small tears in the dura can occur during this procedure, although they are rare, and typically heal within a few days without adverse effect. Should one occur, we will consult with a laboratory animal veterinarian to determine how frequently to open and flush the chamber with saline and how much time should be allowed for the dura to heal before attempting additional scraping or neural recording. We anticipate that it will be necessary to repeat the scraping procedure in recording chambers approximately 6-12 times during a neural recording experiment.

(Step 17) Survival surgery #3. It may not be possible to collect the necessary quantity of neural data in the first hemisphere, for a variety of reasons. Monkeys may not work consistently during periods of head stabilization. The dura may thicken to a point where electrodes break before entering the brain before sufficient neural data has been collected. Microscopic damage to the cerebral cortex caused by repeated electrode penetration may render it difficult to record additional neurons. If necessary to record sufficient neural data from cortical target areas, we will perform a 3rd survival surgery to make craniotomies and implant recording chambers over target cortical areas in the opposite cerebral hemisphere. However, one monkey will only ever experience at most 3 survival surgeries and have 4 chambers, at the maximum, implanted into the skull.

(Step 18) Recovery from surgery #3. As described in Step 10.

(Repeat of Steps 11 – 16 above with neural recording and electrical stimulation in the 2nd cerebral hemisphere)

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D. **Species.** Justify the choice of species for this protocol.
► **Species: Macaca mulatta. Sex: Male or Female. Age/Size: 2-15 years, 3-12 Kg BW.**

Macaques offer the best animal model of human cognition in which it is practical to utilize invasive neural recording to relate cognitive processes to neuronal activity at a cellular level. The degree of structural and functional similarity between the cerebral cortex of macaques and humans is greater than exists between humans and any alternative experimental species in which invasive recording can be performed. This makes it possible to characterize the neural mechanisms responsible for relatively sophisticated cognitive functions shared by human and nonhuman primates, which may not exist, or exist only in rudimentary form, in simpler mammalian species such as cats, dogs, or rodents. The greater anatomical homology of the cerebral cortex and greater similarity in cognitive ability that exists between humans and monkeys, in comparison to other experimental animals, is particularly important for studies that attempt to replicate features of human neuropsychiatric disease in an animal model. Macaques are readily available and have been extensively investigated in cognitive neurophysiological studies, providing the necessary foundation of knowledge to fully interpret the data we will collect. The size of the monkeys we propose to use is appropriate for behavioral training on complex tasks and neurophysiological recording, based on our previous experience using these techniques.

Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.) Qualifications to perform specific procedures: include an approximate number of times the procedure has been performed, including time frame of most recent experience (e.g., has performed >20 retro-orbital bleeds during the last 2 years). **If refresher training is desired please contact the VMU.**

1. PI

Name►[redacted]**, Ph.D., Associate Professor of Neuroscience**
Animal research experience ► **Has more than 20 years' experience**

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
Behavioral manipulations (including chair restraint, operant conditioning with head stabilization)	**More than 20 years' experience and at least 18 monkeys**
Structural MRI and CT acquisition	**More than 10 years' experience (MRI), at least 18 monkeys, and to be trained (CT)**
Pharmacological manipulations (involving administration of NMDAR antagonists)	**More than 5 years' experience, 3 monkeys**
Sterile surgery to prepare monkeys for head stabilization	**More than 20 years' experience, at least 18 monkeys**
Sterile surgery to prepare monkeys for microelectrode neural recording	**More than 20 years' experience, at least 18 monkeys**

Neurophysiological recording using microelectrode arrays	**More than 10 years' experience, at least 14 monkeys**
Implantation of deep brain stimulating probe	**To be trained**
Neurophysiological recording and Neural stimulation using a deep brain stimulating probe	**To be trained**
Neurophysiological recording and microstimulation using microelectrodes	**More than 10 years' experience, at least 10 monkeys**
Application of antimitotic agents to slow thickening of the dura	**To be trained**
Periodic scraping of the dura mater to reduce its thickness	**More than 20 years' experience, at least 18 monkeys**

2. Other research personnel (copy the lines below for each individual who will be responsible for performing any of the experimental procedures on the animals on this protocol)

   Name► [redacted]**, Graduate Student**
   Animal research experience ► **Has 3 years' experience**

   Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Behavioral manipulations (including chair restraint, operant conditioning with head stabilization)	**3 years' experience, 2 monkeys**
Structural MRI and CT acquisition	**1 years' experience, two monkeys**
Pharmacological manipulations (involving administration of NMDAR antagonists)	**2 years' experience, two monkeys**
Sterile surgery to prepare monkeys for head stabilization	**1 years' experience, one monkey**
Sterile surgery to prepare monkeys for microelectrode neural recording	**1 years' experience, one monkey**
Neurophysiological recording using microelectrode arrays	**1 years' experience, one monkey**
Implantation of deep brain stimulating probe	**To be trained**
Neurophysiological recording and Neural stimulation using a deep brain stimulating probe	**To be trained**

Neurophysiological recording and microstimulation using microelectrodes	**To be trained**
Application of antimitotic agents to slow thickening of the dura	**To be trained**
Periodic scraping of the dura mater to reduce its thickness	**To be trained**

Name► [redacted]**, Graduate Student**
Animal research experience ► **Has 1 year experience**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Behavioral manipulations (including chair restraint, operant conditioning with head stabilization)	**1 years' experience, two monkeys**
Structural MRI and CT acquisition	**To be trained**
Pharmacological manipulations (involving administration of NMDAR antagonists)	**To be trained**
Sterile surgery to prepare monkeys for head stabilization	**To be trained**
Sterile surgery to prepare monkeys for microelectrode neural recording	**To be trained**
Neurophysiological recording using microelectrode arrays	**1 years' experience, one monkey**
Implantation of deep brain stimulating probe	**To be trained**
Neurophysiological recording and Neural stimulation using a deep brain stimulating probe	**To be trained**
Neurophysiological recording and microstimulation using microelectrodes	**To be trained**
Application of antimitotic agents to slow thickening of the dura	**To be trained**
Periodic scraping of the dura mater to reduce its thickness	**To be trained**

Name► [redacted]**, Ph.D., Associate Professor of Neuroscience**
Animal research experience ► **Has more than 20 years' experience**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Structural MRI acquisition	**More than 20 years' experience, at least 10 monkeys**
Sterile surgery to prepare monkeys for head stabilization	**More than 20 years' experience, at least 10 monkeys**
Sterile surgery to prepare monkeys for microelectrode neural recording	**More than 20 years' experience, at least 10 monkeys**
Application of antimitotic agents to slow thickening of the dura	**More than 5 years' experience, at least 2 monkeys**
Periodic scraping of the dura mater to reduce its thickness	**More than 20 years' experience, at least 10 monkeys**
Survival surgery, and neural recording in the opposite cerebral hemisphere	**More than 20 years' experience, at least 10 monkeys**

Name► [redacted] **M.D., Professor of Neuroscience**
Animal research experience ► **Has more than 20 years' experience**

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
Structural MRI acquisition	**More than 20 years' experience, at least 20 monkeys**
Sterile surgery to prepare monkeys for head stabilization	**More than 20 years' experience, at least 20 monkeys**
Sterile surgery to prepare monkeys for microelectrode neural recording	**More than 20 years' experience, at least 20 monkeys**
Application of antimitotic agents to slow thickening of the dura	**More than 5 years' experience, at least 2 monkeys**
Periodic scraping of the dura mater to reduce its thickness	**More than 20 years' experience, at least 20 monkeys**
Survival surgery, and neural recording in the opposite cerebral hemisphere	**More than 20 years' experience with microelectrode recording and electrical stimulation, 5 years' experience recording neural activity with chronic cortical electrode arrays, at least 20 monkeys**

Name► [redacted]**, Senior Laboratory Services Coordinator**
Animal research experience ► **Has more than 20 years' experience**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Behavioral manipulations (including chair restraint, operant conditioning with head stabilization)	**More than 20 years' experience, at least 35 monkeys**
Structural MRI and CT acquisition	**More than 10 years' experience (MRI), at least 35 monkeys, and to be trained (CT)**
Pharmacological manipulations (involving administration of NMDAR antagonists)	**More than 3 years' experience, at least 6 monkeys**
Induction and maintenance of anesthesia during sterile surgery to prepare monkeys for head stabilization	**More than 20 years' experience, at least 35 monkeys**
Induction and maintenance of anesthesia during sterile surgery to prepare monkeys for microelectrode neural recording	**More than 20 years' experience, at least 35 monkeys**
Neurophysiological recording using microelectrode arrays	**More than 20 years' experience, at least 35 monkeys**
Implantation of deep brain stimulating probe	**To be trained**
Neurophysiological recording and Neural stimulation using a deep brain stimulating probe	**To be trained**
Neurophysiological recording and microstimulation using microelectrodes	**To be trained**
Application of antimitotic agents to slow thickening of the dura	**More than 5 years' experience, at least 4 monkeys**
Periodic scraping of the dura mater to reduce its thickness	**To be trained**
Induction and maintenance of anesthesia during survival surgery, and neural recording in the opposite cerebral hemisphere	**More than 20 years' experience, at least 35 monkeys**

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual member of the

VMU animal care and veterinary staff who will perform support procedures other than routine husbandry on the animals on this protocol.)

Name►

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)

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course (this refers to the CITI training courses):

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)
Dr. [redacted]	**7/7/14**	**NHP 7/7/14**	
[redacted]	**11/24/14**	**NHP 11/24/14**	
[redacted]	**8/18/14**	**NHP 8/17/14**	
Dr. [redacted]	**1/6/15**	**NHP 1/6/15**	
Dr. [redacted]	**4/6/17**	**NHP 3/13/17**	
[redacted]	**1/23/15**	**NHP 1/16/15**	

F. **Training to be provided.** List here each procedure in Item 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, enter "N/A"
►

1. Structural MRI and CT acquisition). Personnel will be trained how to anesthetize monkeys using ketamine/xylazine, how to fix their head in a MRI/CT compatible stereotaxic apparatus, how to transport monkeys to the MRI or CT facility, how to assist the MRI/CT technician in aligning the head of the monkey with the image acquisition plane in the scanner, how to monitor the depth of anesthesia and administer supplementary doses of ketamine as needed during the scanning procedure, how to return the monkey back to the laboratory, remove the monkey from the stereotaxic apparatus, recover the monkey from anesthesia in a primate chair and return safely to their home cage. Training will be provided by Dr. [redacted] and [redacted] (each with more than 10 years' experience in performing these procedures and training people to perform them). Since the CT scan procedure is very similar to the MRI scan procedure in terms of anesthesia, head stabilization in a stereotaxic frame, and maintenance of the monkey during scan acquisition and afterwards, no hands-on training will be required to obtain CT scans of nonhuman primates at the VA, as we have extensive experience with the MRI scanning procedure in nonhuman primates. We will consult with Dr. [redacted] (Department of Biomedical Engineering, University of [redacted]) once CT scans are acquired to integrate the CT scan data with MRI scan data to achieve accurate 3D localization of recording and DBS sites. Dr. [redacted] has more than 8 years' experience combining MRI and CT data to localize deep brain stimulating sites in nonhuman primates. This consultation will involve how to utilize (but not acquire) the CT scan data.

2. Pharmacological manipulations (involving administration of NMDAR antagonists) Personnel will be trained how to administer NMDAR antagonists via intramuscular injection, and to monitor monkeys for adverse effects. These effects are expected to be minor or absent at the doses we will employ in these studies, but should any occur, personnel will be instructed to consult with a laboratory animal veterinarian immediately to determine whether to administer diazepam (0.5 – 1.0 mg/kg IM) to ameliorate adverse reactions to PCP. Training will be provided by Dr. [redacted] and [redacted] (each with more than 3 years' experience in performing these procedures and training people to perform them).

3. Sterile surgery to prepare monkeys for head stabilization. Personnel will be trained how to: induce anesthesia

with injectable anesthetics, insert intravenous catheters, shave the scalp, perform endotracheal intubation, initiate and maintain gas anesthesia, evaluate the depth of anesthesia, stabilize head position in a stereotaxic frame, disinfect the scalp, make a scalp incision to expose the skull, drill holes for screws in the skull using a high speed hand drill, place posts or blocks over the screw holes and screw them into the skull, surround the posts or blocks with dental acrylic or bone cement, suture the skin so that the wound margin conforms closely to the perimeter of the acrylic/cement, administer appropriate injectable analgesics, remove monkeys from the stereotaxic frame, terminate gas anesthesia, attach a halo or other mechanical anchoring device to the implanted posts or blocks, and recover monkeys so that they regain motor function and can be safely returned to their home cage. Surgical training will be provided by Dr. [redacted] (with more than 20 years' experience in performing these procedures and training people to perform them).

4. Sterile surgery to prepare monkeys for microelectrode neural recording. Personnel will be trained how to: induce anesthesia with injectable anesthetics, insert intravenous catheters, shave the scalp, perform endotracheal intubation, initiate and maintain gas anesthesia, evaluate the depth of anesthesia, stabilize head position in a stereotaxic frame, disinfect the scalp, make a scalp incision to expose the skull, utilize the stereotaxic frame to locate sites for craniotomies and neural recording on the skull, make craniotomies at these locations using a trephine or high speed hand drill, use rongeurs to smooth the margin of the craniotomies, drill holes for screws in the skull surrounding the craniotomies using a high speed hand drill, place screws in the holes, place recording chambers within the craniotomies, and cement the chambers to the screws in the surrounding skull with dental acrylic or bone cement, suture the skin so the wound margin conforms to the perimeter of the cranial implant, administer appropriate injectable analgesics, remove monkeys from the stereotaxic frame and recover monkeys from anesthesia. Surgical training will be provided by Dr. [redacted] (with more than 20 years' experience in performing these procedures and training people to perform them).

5. Neurophysiological recording using microelectrode arrays. Personnel will be trained how to: bolt halos attached to implanted posts on the skull or other mechanical anchoring devices implanted on the monkeys' skull to an external brace to stabilize head position, open and clean recording chambers in preparation for neural recording, position the monkeys comfortably in the recording chamber in the laboratory, position microelectrode arrays within the recording chambers, advance microelectrodes into cortical tissue, isolate and record neural activity, and withdraw the microelectrodes after neural recording, clean and close recording chambers after use, clean the wound margin. In the case of neural recording from subcortical target brain areas (Group 1), personnel will be additionally trained how to puncture the dura with a guide tube, and advance the linear electrode array through the guide tube through the cortex down to the subcortical target. Training will be provided by Dr. [redacted] and [redacted] (each with more than 20 years' experience in performing these procedures and training people to perform them).

6. Implantation of deep brain stimulating probe. Personnel will be trained to drive a sterilized guide tube through the dura to a depth several millimeters (~5-10) above the target subcortical area using a microdrive, insert a sterilized DBS electrode through the guide tube and advance it until the stimulation contacts at the tip of the DBS electrode extend several millimeters below the bottom of the guide tube, remove the guide tube, cement the top of DBS electrode in place to a ring inserted into the recording chamber, attach the wire leads of the DBS electrode to a connector, and position the connector within a closable chamber attached to the cranial implant. To obtain the necessary training in DBS electrode implantation, localization, and maintenance procedures and techniques, we will consult with Dr. [redacted] (Department of Biomedical Engineering, University of [redacted]) who has more than 8 years' experience employing chronically implanted DBS electrodes of the design we will utilize to conduct electrical stimulation of subcortical structures in nonhuman primates. Dr. [redacted] has implanted DBS probes in subcortical targets and studied the effects of brain stimulation on neural activity and behavior in more than 8 nonhuman primates. Overall, this procedure is similar to inserting a recording probe with the differences being the DBS probe's larger outer diameter (0.7 mm) and the chronicity of the probe implant. We have observed the DBS probe implantation procedure in [redacted] lab. Dr. [redacted], will observe and instruct us in the probe implantation procedure (without performing it [redacted] self) at the VA for the first animal. Dr. [redacted] will provide step-by-step oversight and guidance under close observation of the DBS probe implantation procedure at the VA while we insert the DBS probe. Close observation and verbal instruction will be sufficient to oversee and train the procedure, because it is relatively straightforward. It will be helpful to have [redacted] experience and judgment in observing and overseeing our first DBS implantation procedure at the VA, but it will not be necessary for [redacted] to have hands on access to perform the procedure itself, in order to train us in its successful implementation. The reason for this is that the procedure essentially involves three main steps, as follows: (1) Insertion of guide tube. We do this routinely during daily neural recording using linear electrode arrays, and so have experience with the procedure. The guide tube is supported by

the microdrive and it is advanced by rotating a knob on the microdrive. The procedure will be the same for DBS probe implantation. (2) Insertion of DBS probe. Once the guide tube is in place above the brain target for stimulation, the DBS probe is lowered into the guide tube and advanced through the guide tube to the proper depth using the microdrive. The process of advancing the DBS probe is essentially the same as advancing the linear electrode array during neural recording. We have experience with this procedure. (3) Removal of the guide tube and microdrive leaving the DBS probe in place. At this stage, the guide tube is first withdrawn so it is no longer in the brain, and the microdrive is removed, leaving the DBS probe in place. This is a straightforward procedure that observation and close supervision by Dr. [redacted] should be sufficient to ensure is properly performed. After receiving this instruction by Dr. [redacted] for DBS probe implantation in the first animal, we will perform the DBS probe implantation procedure independently in subsequent animals.

7. CT localization of DBS electrodes. Procedures are as described in 'Structural MRI and CT acquisition (Step 4)'.

8. Neural stimulation. Personnel will be trained how to attach stimulator leads to DBS electrodes (Group 1), microelectrodes (Groups 1-3), and how to set the timing and amplitude of small electrical currents delivered by stimulators designed for the stimulation of neural tissue (for electrical stimulation pulse parameters, see Part C.2.c; Step 15, above). Training for DBS stimulation parameters and administration will be provided by Dr. [redacted] (with more than 5 years' experience applying DBS in nonhuman primates). We will observe the DBS stimulation procedure in Dr. [redacted]'s laboratory at the University of [redacted] if the procedure is planned to take place as part of ongoing experiments in that laboratory within the time frame preceding stimulation of the animals in this study. We will consult with Dr. [redacted] in determining the parameters of the DBS stimulation and he will instruct us on the potential signs of adverse effects. Training for microstimulation will be provided by Dr. [redacted] (with more than 10 years' experience using microstimulation in nonhuman primates).

9. Application of antimitotic agents to slow thickening of the dura. Personnel will be trained how to: safely prepare, handle and dispose of dilute 5-fluorouracil solutions, open recording chambers, apply 5-fluorouracil solutions to recording chambers, remove 5-fluorouracil solutions, flush the chambers with saline, and close the chambers. Training will be provided by [redacted] (with more than 5 years' experience in performing these procedures and training people to perform them).

10. Periodic scraping of the dura mater to reduce its thickness. Personnel will be trained how to: sedate monkeys with ketamine, open recording chambers, use surgical tools under an operating microscope to partially dissect and remove outer layers of the dura mater to reduce its thickness, manage any tears in the dura that might occur, flush chambers with sterile saline, close the chambers. Training will be provided by Dr. [redacted] (with more than 20 years' experience in performing these procedures and training people to perform them).

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 OHSP		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program and submit documentation of participation		
Dr. [redacted]	(**X**)	( )	( )	yes
[redacted]	(**X**)	( )	( )	yes
[redacted]	(**X**)	( )	( )	yes
Dr. [redacted]	(**X**)	( )	( )	yes
Dr. [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?

► (**X**) Yes. Describe them ► **Personnel have been trained with regard to zoonotic and reverse zoonotic diseases of primates, such as tuberculosis and herpes B infections.**

► ( ) No.

Animals Requested

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
Macaca Mulatta	**Male/Female**	**2-15 years / 3-12 Kg BW**	**University of [redacted] Medical School, University of [redacted] ([redacted] National Primate Research Center), or University of [redacted] ([redacted] National Primate Research Center)**	**SPF B-Virus Seronegative**

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 / Experimental Group / Procedures(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category B TOTAL**

USDA Category C

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**

USDA Category D

Procedures►

Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**
Macaca Mulatta	**3**	**3**	**2**			**8**

USDA Category E

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
Macaca Mulatta	**3**	**3**	**2**			**8**

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)
Surgical Procedures	**See Appendix 5 for details**	**See Appendix 5 for details**	**See Appendix 5 for details**
Dural scraping	**Monkeys will be visually monitored continuously throughout the procedure and afterwards until fully recovered from anesthesia**	**Dr. [redacted], [redacted], [redacted], [redacted]**	**Anesthesia will be induced by IM injection (2-10 mgs/kg ketamine; or ketamine,7.0 mg / kg, and xylazine 0.6 mg / kg). In addition, meloxicam (0.2mg/kg SQ first day, 0.1mg/kg subsequently) once a day for a minimum of 1 day will be given for analgesia**

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

   Name► **Dr.** [redacted]
   Institutional affiliation► **Minneapolis Veterans Affairs Health Care System, Minneapolis, MN**
   email contact► [redacted]

2. Veterinary consultation during the planning of this protocol (must have occurred within one year of submission).

   Name of the laboratory animal veterinarian consulted► **Dr.** [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ►**6/6/2017**

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.)

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
Macaca Mulatta	**Standard (see SOP)**	**1-2**	**Yes**	**4**

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:
►

** 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)
► At the beginning of training, the monkeys will be pair-housed. As male monkeys mature, the potential for aggressive interaction increases. This will be closely monitored, and if aggressive interaction that carries a potential for significant injury is observed, the monkeys will be separated and housed individually. As per the exceptions contained in the Environmental Enrichment Plan, direct social contact may be terminated once a monkey has received a cranial implant. This is necessary because implanted devices are fragile, and their destruction during an aggressive episode could terminate an experiment prematurely, potentially requiring the sacrifice of the monkey in question. However, if a reliable pattern of non-aggressive interaction between a given pair of monkeys is clearly evident and stable, we will attempt

to continue pair housing after cranial implantation of one or both monkeys unless or until aggressive interactions between the two monkeys become apparent.

***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*.):

a. Species	b. Description of Enrichment*	c. Frequency
Macaca Mulatta	**Standard (see SOP)**	

*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.
►

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.
►

► (**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.
► **The management of chronic cranial implants will be per lab specific SOP.**

► ( ) 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.

► ( ) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed.

► (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.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]	[redacted] **or** [redacted] **or as assigned**	(**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.

Name of Non-VA Facility	Is this facility accredited by	Building	Room

	AAALAC?			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 through non-research areas?	
	Yes	No		Yes – describe method of discreet transport	No
Training, microelectrode recording, neural stimulation, and infrared recording of eye movements	**(X)**	**( )**	**[redacted]9, Rooms [redacted], [redacted], [redacted], [redacted], [redacted]**	**( )**	**(X)**
DBS electrode implantation		**(X)**	**[redacted], [redacted], [redacted], [redacted], [redacted]**	( )	**(X)**
MRI	**( )**	**(X)**	**[redacted], Room [redacted]**	**(X) The monkey is sedated, placed on a wheeled cart, and totally covered with a surgical drape. The cart is wheeled through the tunnel door from [redacted] to the MRI suite, and returned to [redacted] via the same route**	
CT scan		**(X)**	**[redacted], [redacted]**	**(X) The monkey is sedated, placed on a wheeled cart, and totally covered with a surgical**	

				drape. The cart is wheeled through the tunnel door from [redacted] **to the CT suite, and returned to** [redacted] **via the same route**	
Euthanasia		**(X)**	[redacted]**, Room** [redacted]	**( )**	

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")
The brains will be extracted, fixed and processed using conventional histological techniques to visualize the sites of neural recording within the cerebral cortex.	**(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.)
► If any of the following conditions occur, we will consult with a veterinarian on staff to determine if it is appropriate to remove animals from the procedures specified in this protocol or euthanize them: (1) The implant is damaged beyond repair and further use by infection or accident. (2) A monkey loses more than 20% of the body weight it maintained while healthy and working under the water restricted protocol, and this weight loss cannot be quickly reversed by cessation of water deprivation. (3) Disease occurs that imposes considerable discomfort to the monkey and that cannot be effectively treated. The decision to euthanize an animal prior to completion of the experiment will be made in consultation with a veterinarian on staff.

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► **Commercial euthanasia solution (such as Euthasol)** Dose► **86 mg/kg pentobarbital or greater** Route of administration► **Intravenous injection**	**Macaca Mulatta**	(**X**)	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Exsanguination under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Other (Describe) ►		( )	( )	( )

( )	Other (Describe) ►		( )	( )	( )

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:
   ►

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:
   ►

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.
   ► **Sacrificing the animals using an overdose of commercial euthanasia solution (such as Euthasol) will be done by Dr.** [redacted]**,** [redacted]**.** [redacted]**, and** [redacted] **may assist. Dr.** [redacted] **and** [redacted] **each has more than 20 years' experience with euthanasia of monkeys.** [redacted] **and** [redacted] **will be trained in euthanasia procedures by Dr.** [redacted] **and** [redacted]**.**

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.
      ► **The animal should be treated as ABSL-2 hazardous tissue, double wrap the carcass, label it as 'biohazard', place in a refrigerator and contact Dr.** [redacted] **or a member of** [redacted] **staff ASAP**

   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► [redacted]
      Contact Information► **cell:** [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.
      ►

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.

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

Prolonged physical restraint, including chairing		Items:	(**X**)**
Operant conditioning and behavioral training		Items:	(**X**)**
Restricted water protocol		Items:	(**X**)**
Cranial implant maintenance	**Routine Implant and Chamber Cleaning in Non-Human Primates (NHPs) SOP 5**	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.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ►1. Water restriction protocol.
   2. Chair restraint
   3. Head fixation
   4. Cranial implant surgery
   5. Pharmacological manipulation of NMDAR function
   6. Dural scraping
   7. Neural stimulation

   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.

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	**6/2/17**	**1966-present**	**Water restriction protocol**	**Primate, monkey, training, food, reward**	( )	( )	(**X**)	( )

Pubmed	**6/2/17**	**1966- present**	**Chair restraint**	**Primate, monkey, unrestrained, neural recording**	( )	( )	(**X**)	( )
Pubmed	**6/2/17**	**1966- present**	**Head fixation**	**Primate, monkey, unrestrained, neural recording**	( )	( )	(**X**)	( )
Pubmed	**6/2/17**	**1966- present**	**Surgical preparation of monkeys for neurophysiological recording**	**Primate, monkey, noninvasive, neural recording**	( )	( )	(**X**)	( )
Pubmed	**6/2/17**	**1966- present**	**Pharmacological manipulation of NMDAR function**	**Primate, monkey, NMDA, antagonist, neural activity**	( )	( )	(**X**)	( )
Pubmed	**6/2/17**	**1966- present**	**Dural scraping**	**Primate, monkey, dura, neural recording**	( )	( )	(**X**)	( )
Pubmed	**6/2/17**	**1966- present**	**All procedures listed above**	**Primate, monkey, prefrontal, neural, neuron, activity, category learning**	( )	( )	( )	(**X**)
Pubmed	**6/2/17**	**1966- present**	**All procedures listed above**	**Primate, monkey, prefrontal, neural, neuron, activity, working memory**	( )	( )	( )	(**X**)
Pubmed	**6/2/17**	**1966- present**	**All procedures listed above**	**Primate, monkey, prefrontal, neural, neuron, activity, executive control**	( )	( )	( )	(**X**)
Pubmed	**6/2/17**	**1966- present**	**All procedures listed above**	**Rodent, rat, prefrontal, neural, neuron, activity**	(**X**)	( )	( )	( )
Altbib	**6/2/17**	**2000-present**	**Neural recording**	**Primate monkey neural activity schizophrenia**	(**X**)	( )	(**X**)	( )
Altbib	**6/2/17**	**2000-present**	**Use of NMDAR antagonists**	**Primate monkey NMDA**	(**X**)	( )	(**X**)	( )
PrimateLit	**6/2/17**	**1940-present**	**Neural recording**	**Neural recording**	(**X**)	( )	(**X**)	( )
PubMed	**6/2/17**	**1966- present**	**Neural Stimulation**	**Primate Deep brain Stimulation, DBS, Primate microstimulation,**	( )	( )	(**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.
► The objective of this research is to understand how brain cells mediate cognition, both in health and disease. This requires experimental conditions that can relate cognition to cellular activity. Single neuron recording in humans is feasible, and is done occasionally in conjunction with brain mapping of

epileptic foci prior to neurosurgical resection. However, it is not ethical to obtain single neuron recordings in humans in circumstances where it is not clinically necessary. Therefore, one replacement in this protocol is to use a less sentient species, monkeys, rather than humans, to achieve the scientific objective. This is feasible because some of the cognitive processes of interest, such as working memory, cognitive control, and categorization exist in nonhuman primates in forms that are analogous to their human counterparts. Further replacement with a less sentient species, such as rodents, is not feasible, because these cognitive processes do not exist in rodents in forms that are directly analogous to their human counterparts. Replacements of invasive neural recording with computational modeling have not been adopted in this protocol because it would not be possible to achieve the scientific objectives of the research with computational modeling alone. Computer modeling studies can neither confirm nor refute hypotheses regarding how electrical signals in cortical neurons mediate cognition. There are several reasons. Computer modeling has not progressed to the point where the results are conclusively predictive of biological results. Models are generally underconstrained, meaning that a large number of models built on very different principles could theoretically produce any behavior of interest, making it impossible to determine which of the model algorithms is actually implemented in the brain. To find out how neural systems process information to support cognition at a cellular level, invasive neural recording is required. Replacements of invasive neural recording with neural recording in non-animal systems such as neuronal cultures has not been adopted in this protocol because it would not be possible to relate neural activity to cognition in this context.

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.
► This protocol utilizes four monkeys per experimental group. This will allow us to test the replicability of neurophysiological findings and drug responses across individual animals. Replicability is an issue of increasing concern in biomedical research. Because our units of analysis and statistical hypothesis testing are individual neurons, the size of our data sample is much larger than the number of animals we test, and we will not perform statistical tests at the group comparison level as is typical in rodent research. However, given that prefrontal neurons and prefrontal networks may respond differently to NMDA receptor blockade in different individual monkeys, it will be important to study how NMDA receptor blockade changes neural and behavioral function in as many individual monkeys as can be practically achieved within the time frame of this ACORP. Four monkeys per experimental group, rather than two monkeys (which is practical minimum number for publication of results in scientific journals), will help mitigate individual variability in neuronal responses, and provide results that are more likely to generalize across monkeys and therefore be replicable. .

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
► This work incorporates several refinements. Recording of eye position is done using noninvasive video eye tracking, rather than surgical implantation of scleral search coils, a previously common technique. Sterile surgery is performed under gas anesthesia conditions comparable to those employed for human surgery. Post-surgical pain is managed using injectable analgesics that provide prolonged pain relief. The psychological well-being of nonhuman primates is supported by pair housing, and environmental enrichment (including time in pairs in room-sized enclosures that allow free movement, pioneered by the research, veterinary and animal care staff at the Minneapolis VA). Advanced multi-electrode arrays are utilized to maximize the amount of neural data that can be obtained from each craniotomy, potentially minimizing the number of craniotomies necessary to obtain enough neural recordings to answer the research questions of interest. Every effort will be made to minimize the number of survival surgeries needed to obtain a neuronal database of sufficient size, potentially to one per animal (although this will depend on experimental factors that govern how much data it is possible to collect in each animal which are difficult to predict beforehand). The technology to refine the neural recording method further to acquire the data needed in a way that is less invasive does not yet exist. Chronically implanted microelectrode arrays for single neuron recording to replace the acute microelectrode arrays we employ would lessen stress on the animal by making it unnecessary to open and clean recording chambers. However, they cannot acquire recordings from enough neurons yet to answer the research questions in this proposal. Therefore, refinement of the recording method is not yet feasible. Lastly, deep brain stimulating probes are the same probes used in humans, only scaled down

versions and therefore are well known to effectively stimulate brain regions without damaging the brain or harming the subject.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
► **An examination of a PUBMED literature search (1966-present) with search terms covering the field of single neuron recording and stimulation in monkey prefrontal networks (terms: primate, monkey, prefrontal, neural, neuron, activity, DBS, mediodorsal nucleus of the thalamus(MD)) revealed that the proposed research will not duplicate previous work. No prior studies in monkeys have utilized the specific working memory and executive control tasks that we will use, selected because they are known to measure specific cognitive impairments in patients with neuropsychiatric disease (such as schizophrenia). The planned experiments to characterize the neural basis of category learning are novel. No prior study has investigated the role of NMDA receptor function in relation to these cognitive tasks at a combined cellular and network level in nonhuman primates. No prior studies have stimulated in the mediodorsal nucleus of the thalamus or other subcortical structures in monkeys using a deep brain stimulation protocol during performance of a cognitive control task or as a remediation for deficits induced by NMDAR antagonists as a potential therapeutic for cognitive deficits in psychiatric disorders.**

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

a. 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 Phar-macy	Non-VA
Ketamine	(**X**)	( )*	**Dr.** [redacted]	(**X**)	( )	(**X**)	( )
Buprenorphine (regular or SR)	(**X**)	( )*	**Dr.** [redacted]	(**X**)	( )	(**X**)	( )
Phencyclidine	(**X**)	( )*	**Dr.** [redacted]	(**X**)	( )	(**X**)	( )
Commercial euthanasia solution	(**X**)	( )*	**Dr.** [redacted]	(**X**)	( )	(**X**)	( )
Diazepam	(**X**)	( )*	**Dr.** [redacted]	(**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.
►

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

► (X) Some controlled substances will be 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?

   ► (X) 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".

   ► ( ) 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 include (i.e., delete) any appendices that are not applicable to this ACORP.

► ( ) 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"
► (**X**) 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*"

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* If referencing SOPs specific to your lab you MUST ensure that the IACUC office is supplied with the most current version for IACUC review at the time of submission or amendment.**

Item	SOP		IACUC Approval Date*
	Title	ID	
M.1	**Receiving Nonhuman Primates**	**HUSB-102**	10/27/2016
M.1	**Daily Feeding, Watering, and Providing Food Environmental Enrichment for Nonhuman Primates**	**HUSB 122**	10/27/2016
M.1	**Changing and Cleaning Nonhuman Primate Cages**	**HUSB 132**	10/27/2016
M.1	**Daily Health Checks for Large and Small Laboratory Animals**	**HUSB 150**	10/27/2016
U.4.b	**Handling and Processing Animals Found Dead**	**OPR-205**	10/27/2016
V	**Routine Implant and Chamber Cleaning in Non-Human Primates (NHPs) SOP 5**	**BSC-5**	10/20/2016

***Current SOPs and corresponding IACUC Approval Dates are kept on file in the IACUC Office and may**

supersede those listed here.

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.

Name(s) of Principal Investigator(s)	Signature	Date
[redacted]**, Ph.D.**		

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] **D.V.M.**		
Name of IACUC Chair	Signature	Date
[redacted] **Ph.D.**		

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.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]**, Ph.D.**		
Name of Institutional Veterinarian	Signature	Date
[redacted] **D.V.M.**		

Name of IACUC Chair	Signature	Date
[redacted] **Ph.D.**		

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] **Ph.D.**		

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
[redacted]		

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.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]**, Ph.D.**		

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.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]**, Ph.D.**		

   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
[redacted] **Ph.D.**		
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
[redacted] **Ph.D.**		
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted] **D.V.M.**		
Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment	Signature	Date
[redacted] **M.D.** **Director, Radiation Oncology (CT)**		
[redacted] **M.D.** **Director, Imaging Product (MRI)**		
Name of ACOS for R&D	Signature	Date
[redacted] **M.D.**		
Name of Chief of Staff	Signature	Date
[redacted] **M.D.**		
Name of Director or CEO of the Facility (Hospital or Clinic)	Signature	Date
Patrick J. Kelly, FACHE		

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).

Name(s) of Principal Investigator(s)	Signature(s)	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
[redacted] **Ph.D.**		
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted] **D.V.M.**		
Name of Safety/Biosafety Officer for the Facility	Signature	Date
[redacted]		
Name of ACOS for R&D	Signature	Date
[redacted] **M.D.**		

Name of VISN Regional Safety Officer	Signature	Date

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

ACORP APPENDIX 3
BIOSAFETY
VERSION 4 MPLS VAHCS NOV 2013

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 agent, device, strain, construct, isotope, etc.)	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**						
		Toxic Agent (Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural □Drug	Euthanasia agent
5-fluorouracil	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	**(X)**	( )
Ketamine	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Phencyclidine hydrochloride	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	()	( )
Buprenorphine	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Buprenorphine (sustained release)	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Dexamethasone	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	**(X)**	( )
Xylazine	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Atropine	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Isoflurane	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	**(X)**	( )
Baytril	Bayer	()	( )BSL_	( )	( )	( )	**(X)**	( )
Meloxicam	Henry Schein	()	( )BSL_	( )	( )	( )	**(X)**	( )
Acrylic Resin Powder/Liquid	Lang Dental	**(X)**	( )BSL_	( )	( )	( )	**( )**	( )
Commercial Surgical Bone Cement	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	**( )**	( )
Commercial euthanasia solution (Euthasol)	VA Pharmacy	**(X)**	( )BSL_	( )	( )	( )	**( )**	**(X)**

Diazepam Injection	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Biocompatible foam (Gelfoam)	VA Pharmacy	()	( )BSL_	( )	( )	( )	( )	( )
Biocompatible film (Gelfilm)	VA Pharmacy	()	( )BSL_	( )	( )	( )	( )	( )
ChloraPrep with Tint	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Lidocaine HCl 1% & Epinephrine 1:100,000 Inj.	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )
Biocompatible posts and blocks	VA Medical Instrumentation Shop	()	( )BSL_	( )	( )	( )	()	( )
Plastic recording chambers	VA Medical Instrumentation Shop	()	( )BSL_	( )	( )	( )	()	( )
Saline	VA Pharmacy	()	( )BSL_	( )	( )	( )	()	( )
Cefazolin	VA Pharmacy	()	( )BSL_	( )	( )	( )	**(X)**	( )

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:

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)
5-fluorouracil	**2.5% solution ~1mL**	**Sterile Saline**	**Solution applied to surface of dura mater within recording chambers**	**5-10 minutes daily, 5 days a week**	**Antimitotic agent used to slow growth of dura, will facilitate electrode penetration**	**Main body**	**N**
Ketamine	**0.05 to 10 mg/kg, 0.0005-0.1 mL/kg**	**Sterile Saline**	**IM**	**Once a day, for up to 6 weeks**	**Reduce NMDA receptor function, and to induce anesthesia**	**Main body**	**N**

Phencyclidine hydrochloride*	**0.15-0.3 mg/kg, 0.05-0.1 mL/kg**	**Sterile Saline**	**IM**	**Once every other day, for up to 6 weeks**	**Reduce NMDA receptor function**	**Main body**	**N**
Buprenorphine	**0.005- 0.05 mg/kg, 0.017-0.07 mL/kg**	**Sterile Saline**	**IM, IV**	**2 / day, up to 3 days post-operative**	**Manage pain**	**Appendix 5**	**N**
Buprenorphine (sustained release)	**0.15 - 0.25 mg/kg, 0.015-0.025 mL/kg**	**Sterile Saline**	**SC, IM**	**1 / 72 hrs**	**Manage pain**	**Appendix 5**	**N**
Dexamethasone	**0.5 mg / kg, 0.125 mL/kg**	**Sterile Saline**	**IM**	**As directed by veterinarian**	**Reduce cortical swelling**	**Appendix 5**	**N**
Xylazine	**0.5-1 mg / kg, 0.025-0.05 mL/kg**	**Sterile Saline**	**IM**	**During each survival surgery (up to 3 per animal) and MRI scanning**	**Induce anesthesia**	**Appendix 5, 6**	**N**
Atropine	**0.05-0.1 mg / kg, 0.125-0.25 mL/kg**	**Sterile Saline**	**IM**	**During each survival surgery (up to 3 per animal)**	**Reduce secretions**	**Appendix 5**	**N**
Isoflurane	**1 - 4%**	**Gas (oxygen)**	**Endotracheal intubation**	**Continuously during surgery**	**Induction of surgical anesthesia**	**Appendix 5**	**N**
Baytril	**4 mg/kg, 0.04 mL/kg**	**Sterile Saline**	**IM**	**As directed by veterinarian**	**Antibiotic**	**Appendix 5**	**N**
Meloxicam	**0.2 mg/kg, 0.04 mL/kg (1st dose), then 0.1 mg/kg, 0.02 mL/kg (ea. subsequent dose)**	**Sterile Saline**	**SC**	**1/day, up to 3 days post-operative**	**Manage pain**	**Appendix 5**	**N**
Acrylic Resin Powder/Liquid*	**N/A**	**N/A**	**Surgical application o surface of skull**	**During each survival surgery (up to 3 per animal)**	**Anchor cranial implant**	**Appendix 5**	**Y**

Surgical Bone Cement*	**N/A**	**N/A**	**Surgical application on surface of skull**	**During each survival surgery (up to 3 per animal)**	**Anchor cranial implant**	**Appendix 5**	**Y**
Euthasol Euthanasia Soln.	**86 mg/kg, 1 mL/4.5 kg pentobarbital**	**Sterile Saline**	**IV**	**Once at termination of experiment**	**Euthanize animal**	**Main body**	**Y**
Diazepam Inj.	**0.5 – 1.0 mg/kg, 0.1-0.2 mL/kg**	**Sterile Saline**	**IV, IM, or rectally**	**As directed by veterinarian**	**Reduce potential adverse effects of NMDA antagonists**	**Main Body**	**N**
Lidocaine HCl 1% & Epinephrine 1:100,000 Inj.	**5-10 mg/mL Lidocaine & 5-10 mcg/mL Epinephrine (0.5-1.0 mL)**	**Sterile Saline**	**Percutaneous infiltration along line of planned incision**	**During first survival surgery**	**Local anesthetic / vasoconstriction**	**Appendix 5**	**Y**
Biocompatible posts and blocks	**N/A**	**N/A**	**Implantation on skull during aseptic surgery**	**Once during first survival surgery**	**Enable stabilizing head fixation for neural recording**	**Appendix 5 Main Body (C.2.c)**	**Y**
Plastic recording chambers	**N/A**	**N/A**	**Implantation on skull during aseptic surgery**	**Once during second and third survival surgeries**	**Cover craniotomies and allow access for neural recording**	**Appendix 5 Main Body (C.2.c)**	**Y**
Saline	**10-20 ml/kg/hr**	**N/A**	**IV**	**Continuous during surgery**	**Replenish fluids maintain hydration**	**Appendix 5**	**Y**
Cefazolin	**20-25mg/kg**	**Sterile Saline**	**IV**	**Once every 2 hours**	**Antimicrobial prophylaxis**	**Appendix 5**	**Y**
Biocompatible foam (Gelfoam)	**N/A**	**N/A**	**Applied to surface of dura within recording chamber**	**Once per dural tear**	**To facilitate healing of the dura**	**Main Body (C.2.c)**	**Y**
Biocompatible film (Gelfilm)	**N/A**	**N/A**	**Applied to surface of dura within recording chamber**	**Once per dural tear**	**To facilitate healing of the dura**	**Main Body (C.2.c)**	**Y**

ChloraPrep with Tint	**N/A**	**N/A**	**Applied to scalp before surgical incision**	**Once per survival surgery**	**Disinfect skin**	**Appendix 5**	**Y**

*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.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and 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.)

► **1. Phencyclidine hydrochloride (PCP).** Phencyclidine hydrochloride is not available in a pharmaceutical grade preparation. The material will be made appropriate for intramuscular injection prior to injection, with consideration of appropriate pH (4.5-8) and sterility. The IM injection site will be rotated to help further avoid discomfort. For preparation of PCP injection solutions, we follow the procedure of Roth and colleagues, who also used a 3.0 mg/ml solution of PCP dissolved in sterile saline (Jentsch JD, Taylor JR, Elsworth JD, Redmond DE, Roth RH. Altered frontal cortical dopaminergic transmission in monkeys after subchronic phencyclidine exposure: involvement in frontostriatal cognitive deficits. Neuroscience 90: 823-832, 19999). Phencyclidine hydrochloride will be obtained as a white crystalline powder. Although it has limited solubility in water at 25ºC, solutions of up to 11 mg/ml can be obtained by dissolving the powder in warmed water. (Tocris Pharmaceutical Certificate of Analysis/Product Data Sheet and website). We will use this procedure to dilute PCP in sterile saline to a concentration of 3.0 mg / ml. After cooling the solution to room temperature, we will then filter the solution with a 0.2 µm filter to remove bacteria prior to injection. The compound will be labeled with an expiration date of no later than 28d from time of reconstitution.

2. Surgical Bone Cement or Acrylic Resin Power/ Liquid. Surgical bone cement will be used to construct the cranial implant, and attach recording chambers to screws and posts fixed to the surface of the skull. Acrylic resin powder/liquid is an alternative material for this purpose that has a lower setting temperature but is not available in a pharmaceutical grade preparation. Acrylic resin powder/liquid is routinely used in cranial implant surgeries in nonhuman primates without post-surgical infection.

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):

► **Bone cement or acrylic resin. Bone cement or acrylic resin will be administered during a sterile surgery under gas anesthesia (1-4% Isoflurane).**

Euthasol. Euthasol will be administered to euthanize animals via intravenous catheter after first anesthetizing animals with the maximum dose of ketamine (doses above).

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.

► **Compounds administered before neural recording (5-fluorouracil, Phencyclidine, Ketamine). 5-fluorouracil will not induce pain or distress. Phencyclidine hydrochloride and ketamine are themselves anesthetics, prior treatment with additional anesthetics is not indicated. IM injections of phencyclidine hydrochloride and ketamine will be administered while monkeys are seated in a primate chair.**

Compounds that are administered to induce anesthesia or as adjuncts to anesthesia (Xylazine, Atropine, ketamine, Isoflurane). These compounds are given to induce anesthesia, or as adjuncts to anesthesia, so no prior anesthesia is indicated or necessary. IM injections of xylazine and atropine will be administered while monkeys are seated in a primate chair. Isoflurane is a gas anesthetic and will be administered via

endotracheal intubation.

<u>Compounds that are administered to manage pain or fight infection (Buprenorphine, Buprenorphine sustained release, Meloxicam, Baytril).</u> These compounds are analgesics or antibiotics. They are administered by minimally stressful IM or SC injection. IM or SC injections of these compounds will be administered while monkeys are seated in a primate chair.No prior anesthesia is indicated.

<u>Compounds that are administered to reduce cortical swelling (Dexamethasone).</u>
This compound will be administered at the direction of a laboratory animal veterinarian to reduce cortical edema if indicated. Administration is by minimally stressful IM injection. IM injections will be administered while monkeys are seated in a primate chair or intraoperatively while the animal is anesthetized. No prior anesthesia is indicated.

<u>Compounds that are administered to reduce potential adverse effects of NMDA receptor antagonists (Diazepam).</u> This compound will be administered at the direction of a laboratory animal veterinarian to reduce potential adverse effects of NMDA receptor antagonists. Administration is by minimally stressful IM injection. IM injections will be administered while monkeys are seated in a primate chair. No prior anesthesia is indicated.

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 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
				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	
5-fluorouracil	( )	**(X)**	( )	**(X)**	( )	( )*	( )►
Phencyclidine hydrochloride	( )	( )	**(X)**	**(X)**	( )	( )*	( )►
Dexamethasone	( )	( )	**(X)**	**(X)**	( )	( )*	**(X)► Considered a reproductive toxin. Passes into breast milk of nursing mothers.**
Isoflurane	( )	( )	**(X)**	**(X)**	( )	( )*	( )►
Acrylic Resin Powder/Liquid	( )	( )	**(X)**	**(X)**	( )	( )*	**(X)►Possible teratogen**
Surgical Bone Cement	( )	( )	**(X)**	**(X)**	( )	( )*	**(X)►Possible teratogen**
ChloraPrep Surgical Prep Applicator	( )	( )	**( )**	**(X)**	( )	( )*	**(X)► Serious eye irritant**.
Commercial euthanasia solution (such as Euthasol)	**(X)**	**(X)**	**(X)**	**(X)**	( )	( )*	**(X)►Phenytoin sodium is a carcinogen. Phenobarbital may affect fetus.**

*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)	( )	( )	( )**

*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
NMDA receptor antagonists (ketamine, and phencyclidine hydrochloride)	**Adverse behavioral reactions to NMDA receptor antagonists such as phencyclidine hydrochloride (PCP) can include abnormal motor behaviors (ataxia, dystonia), rhabdomyolysis, cardiac arrhythmias (specifically tachycardia), irregular breathing (Bey and Patel, 2007), and exaggerated reactions to acute stressors (Linn et al., 1999),**	**These reactions are expected to be minor and quickly resolve, or to be absent altogether, at the doses administered in this protocol. However, during and after administration of NMDA antagonists, monkeys will be closely monitored, and if these adverse reactions occur, we will immediately consult with a laboratory animal veterinarian to determine whether to administer diazepam (0.5 – 1.0 mg/kg) acutely to mitigate these adverse effects. We will also consult with a laboratory animal veterinarian to determine whether administration of an anti-arrhythmic drug (such as lidocaine) is clinically indicated. Finally, we will consult with the veterinarian to determine whether to lower the dose of PCP for subsequent studies, switch to a less potent NMDAR antagonist (ketamine), or terminate NMDAR**

		antagonist treatment altogether.

REFERENCES

Bey T, Patel A (2007) Phencyclidine intoxication and adverse effects: A clinical and pharmacological review of an illicit drug. Calif. J. Emerg. Med. 7: 9-14.

Linn GS, O'Keeffe RT, Schroeder CE, Lifshitz K, Javitt DC (1999) Behavioral effects of chronic phencyclidine in monkeys. Neuroreport 10:2789-2793.

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
5-fluorouracil	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Phencyclidine hydrochloride	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Dexamethasone	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Isoflurane	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Acrylic Resin Powder/Liquid	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Surgical Bone Cement	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]
Euthasol Euthanasia Solution	**Subcommittee on Research Safety (SRS)**	**VA**	**Dr.** [redacted]

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 hazards of exposure to the above compounds will be assessed by the VA Biosafety committee if this ACORP is approved. Any hazard to animal facility staff will be via excreted drug or metabolites. Hazards that are posed by this route of exposure as determined by the Biosafety committee will be thoroughly discussed with the above personnel.

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

ACORP Appendix 5
SURGERY
VERSION 4 MPLS VAHCS MAY 2017

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. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	**Implantation of head restraint devices**	( )	( )	( )	(X)*
2	**Perform craniotomies and implant recording / stimulation chambers in first cerebral hemisphere**	( )	( )	( )	(X)*
3	**Perform craniotomies and implant recording / stimulating chambers in second cerebral hemisphere**	( )	( )	( )	(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 protocol in 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:
► To measure neural activity using microelectrodes and linear electrode arrays, we must stabilize the position of the monkey's head. For that reason, after initially training monkeys to perform cognitive tasks, we have to implant devices onto the surface of the skull (biocompatible posts or blocks) to provide mechanical anchors for head restraint. We will do that in the first surgery. After the first surgery we will continue to train monkeys to perform cognitive tasks with head fixation. Once they become proficient, we may perform the second surgery to prepare monkeys for neural recording and stimulation. In the second surgery, we will place 1-3 craniotomies over target brain areas and utilize them for neural recording and/or neural stimulation. Once we make these craniotomies, the dura mater within them thickens with time. Within a period of weeks, the dura can become so thick that microelectrodes break before entering the brain. Also, repeated electrode penetration produces accumulating damage to the underlying cortical tissue. This typically does not produce a visible lesion of the cortex or produce overt changes in behavior, but it can make it increasingly difficult to isolate neural activity as the period of recording progresses. Both factors limit the time during which it is possible to collect neural activity. Once microelectrode recording in cortical target areas is no longer readily feasible, if required to collect the needed amount of neural data, we will perform a third surgery, in which we will make additional craniotomies not to exceed 4 craniotomies in any individual monkey and implant recording chambers on the opposite side of the skull, so that we can record neural activity in the opposite cerebral hemisphere. This will allow us to increase the amount of neurophysiological data we can obtain from each monkey, which in turn reduces the number of monkeys needed to complete the study. Depending on the nature of the task, the distribution of the target brain areas, the size of the craniotomies necessary to reach these targets, and duration of behavioral training with head fixation anticipated, it may be possible to implant head restraint devices and recording chambers over all target brain areas in one surgery, reducing the number of survival surgeries required to meet the experimental objectives.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
► Interval between multiple surgeries: 4 weeks minimum. This is the minimum time required to collect neural activity, or to train monkeys to perform cognitive tasks with head restraint.

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 ► **Anesthesia is induced with ketamine (7.0 mg / kg) and xylazine (0.6 mg / kg). The scalp is shaved with electric clippers, and then with a razor. An intravenous catheter is placed in the saphenous vein, and a sterile saline drip initiated. An endotracheal tube is inserted into the trachea, and anesthesia is continued under isoflurane gas (1 - 4%). Once sufficient anesthesia is achieved, monkeys are placed in a stereotaxic apparatus, on top of a water-circulating heat pads to help maintain normal body temperature. The monkey and stereotaxic frame are covered in sterile drapes, a hole cut over the scalp, and the scalp is disinfected using a ChloraPrep applicator and then allowed to dry. After a scalp injection of Lidocaine HCl and epinephrine, we make a midlateral incision in the scalp, or two incisions in a cross pattern, to expose the skull. We remove layers of connective tissue covering the skull, and use a surgical drill to make several small holes in the skull. We then screw head restraint devices (posts or blocks made of biocompatible material) to the skull. Bone cement or dental acrylic is placed around the restraint devices and over the screws to mechanically strengthen their attachment to the skull, and the wound margin is partially closed so that it conforms to the margin of the acrylic/cement implant using simple interrupted sutures (3-0 nonabsorbable suture material). If needed, sutures will be removed 7-14 days later.**

Surgery 2 ► **Anesthesia, intravenous catheterization, endotracheal intubation, placement in a stereotaxic apparatus, and scalp disinfection and incision are as described above. We will surgically prepare the monkeys for microelectrode neural recording in the prefrontal cortex and another brain area. We will make up to 3 craniotomies: one over the prefrontal cortex, and up to two additional craniotomies over connected brain structures. We will place screws in the skull surrounding the craniotomies and then apply bone cement or dental acrylic to anchor recording chambers to screws and posts. We will partially close the wound margin so that it conforms to the margin of the acrylic/cement using simple interrupted sutures (3-0 nonabsorbable suture material). If needed, sutures will be removed 7-14 days later. As noted above, Surgeries 1 and 2 will be combined into a single surgery if possible (depending on the time required to train monkeys to perform a given task with head fixation before neural recording can commence).**

Surgery 3 ► **Same steps as in Surgery 2 above, except that we make craniotomies and implant recording chambers over prefrontal cortex and connected brain areas in the opposite cerebral hemisphere, up to but not exceeding 4 craniotomies per animal In general, in cases that we conduct neural recording and/or neural stimulation in the left and right cerebral hemispheres, we will make two craniotomies per hemisphere to target prefrontal cortex and a connected cortical or subcortical brain structure bilaterally. An exception might be the case in which we target a deep structure for bilateral DBS to optimize behavioral effects, and a unilateral prefrontal chamber to measure changes in neural activity (for a total of 3 craniotomies and chambers).**

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

Name	Surgery #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	**1-3**	(**X**)	( )	( )	( )
[redacted]	**1-3**	( )	( )	(**X**)	( )
[redacted]	**1-3**	(**X**)	(**X**)	( )	( )
[redacted]	**1-3**	(**X**)	(**X**)	( )	( )

[redacted]	**1-3**	(**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
Bldg [redacted]	**Surgical Suites, room** [redacted]	**1-3**	(**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	**(X) – 24 hours**	**( ) --**	**(X) – Saphenous vein at the posterior aspect of the calf**	**(X) – Endotracheal intubation, placement of the monkey in a stereotaxic frame for head immobilization**
2	**(X) – 24 hours**	**( ) --**	**(X) – Saphenous vein at the posterior aspect of the calf**	**(X) – Endotracheal intubation, placement of the monkey in a stereotaxic frame for head immobilization**
3	**(X) – 24 hours**	**( ) --**	**(X) – Saphenous vein at the posterior aspect of the calf**	**(X) – Endotracheal intubation, placement of the monkey in a stereotaxic frame for head immobilization**

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)
Ketamine	**1-3**	**7.0 mg / kg & 0.07 ml / kg**	**IM**	**1/day**	**1 day**
Xylazine	**1-3**	**0.6 mg / kg & 0.3 ml / kg**	**IM**	**1/day**	**1 day**
Atropine	**1-3**	**0.05 mg / kg & 0.12 ml / kg**	**IM**	**1/day**	**1 day**

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 ► **The hair on the scalp will be clipped with an electric shaver. The scalp will then be shaved with a razor. Once an IV catheter and endotracheal tube are placed, and the animal is placed in the stereotaxic frame, the scalp will be disinfected using a ChloraPrep applicator and then allowed to dry. The animal will then be entirely covered with disposable sterile drapes, and a small opening in the drape cut to allow access to the scalp.**

Surgery 2 ► **Same as Surgery 1**

Surgery 3 ► **Same as Surgery 1**

6. **Intra-operative management.**

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

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Isoflurane (gas)	( )*	**1-3**	**1 - 4%**	**Endo-tracheal Intubation**	**Continuous during surgery**
Saline (0.9%)	( )*	**1-3**	**10-20 ml/kg/hr**	**IV**	**Continuous during surgery**
lidocaine (1%)	( )*	**1-3**	**dose not to exceed 2mg/kg, volume approximately 2ml for a 10kg NHP**	**SQ/intraincisional**	**once**
Cefazolin		**1-3**	**20-25mg/kg**	**IV**	**Once every 2hrs during surgery**

* 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.).
► The monkey will be positioned in a "Bair Hugger" on top of a heating pad. The heating pad uses circulating water to reduce the possibility of burns.

c. **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 will be judged sufficient when the palpebral blink reflex is absent, and when monkeys are unresponsive to mildly painful stimuli such as produced by pinching the skin between the toes. A relaxed muscle tone as measured by low resistance to movement of the jaw is another indicator of sufficient anesthesia assessed just before endotracheal intubation. Sufficiently deep anesthesia is easily maintained so that the monkey is immobile throughout the procedure, and unresponsive to pain. The monkeys breathing rate, heart rate, and EKG are constantly monitored. The blood oxygenation level is also monitored using a pulse oximeter. Non-invasive blood pressure and end tidal C02 may also be monitored.

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	**6-24 months (or a period necessary to complete behavioral training, neural recordings and initial data analysis).**	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	( )*
2	**6-24 months (or a period necessary to complete neural recordings and initial data analysis).**	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	( )*
3	**6-24 months (or a period necessary to complete neural recordings and initial data analysis).**	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	(**X**)	( )*

* Describe any "other" measures to be taken to maintain sterility during surgery.
►To make holes for screws in the skull, we use sterile drill bits and cover the flexible shaft and handle of the high speed surgical drill in a sterilized sheath. The surgeon holds and manipulates the drill only through the sterile sheath to drill the holes.

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

Surgery 1 ► **Heating pads, heat lamp and blankets.**

Surgery 2 ► **Heating pads, heat lamp and blankets.**

Surgery 3 ► **Heating pads, heat lamp and blankets.**

Surgery 4 ►

c. Post-operative analgesia. Complete the table below for each survival 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, 2, 3	**Buprenorphine** **OR** **SR Buprenorphine** **AND** **Meloxicam**	**0.01-0.05 mg/kg & 0.17 ml / kg** **0.15 - 0.25 mg/kg & 0.4 ml** **0.2 mg/kg (1st dose), then 0.1 mg/kg (ea. subsequent dose)**	**IM, IV** **SC** **SC**	**2 / day** **1** **1 / day**	**up to 3 days** **1 inj. per 72 hours** **up to 3 days**

*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)
1-3	**Dexamethasone (if needed to control cortical edema)**	**0.5 mg / kg & 0.12 ml / kg**	**IM**	**1 / day**	**1 day**
1-3	**Baytril**	**3.75 mg / kg & 0.16 ml / kg**	**IM**	**2 / day**	**In consult with the**

					veterinarian 1 to 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)
1-3	**Continuous**	**Until recovered**	**Dr.** [redacted] **,** [redacted]**,** [redacted]**, or** [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)
1-3	**1-2 times daily**	**3-5 days**	**Dr.** [redacted] **,** [redacted]**,** [redacted]**, or** [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 ► **The monkey may open the wound by removing sutures, in which case it would be necessary to briefly sedate the animal with Ketamine (5-10 mg / kg) and resuture the wound. Post-surgical infection may appear surrounding the implanted devices. In this case a veterinarian on staff will be consulted and his recommendations implemented regarding antibiotic administration. It could occur that after survival surgeries #1 the implanted posts, or after surgeries #2 or #3, the recording chambers become loose or damaged after they are implanted, requiring repair, which can necessitate anesthetizing the monkey via the procedures described above to place additional titanium screws in the skull, replace damaged head restraint devices or recording chambers, and/or add additional bone cement to increase the mechanical strength of the cranial implant.**

Surgery 2 ► **Same as for Surgery 1 above**

Surgery 3 ► **Same as for Surgery 1 above**

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

Surgery 1 ► **If any of the following conditions occurs, we will consult with a veterinarian on staff to determine if it is appropriate to remove animals from the protocol or euthanize them: (1) The implant is damaged beyond repair and further use by infection or accident. (2) A monkey's health deteriorates to a point where it stops eating for a period exceeding 5 consecutive days. (3) A monkey loses more than 20% of the body weight it maintained while healthy and working under the water deprivation protocol, and this weight loss cannot be quickly reversed by cessation of water deprivation. (4) Disease occurs that imposes considerable discomfort to the monkey and that cannot be effectively treated. The decision to euthanize an animal prior to completion of the experiment will be made in consultation with a veterinarian on staff**

Surgery 2 ► **Same as for Surgery 1 above**

Surgery 3 ► **Same as for 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 euthanized instead.)
► Any drug deemed appropriate to treat an emergency medical situation by qualified personnel may be used.

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will be 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 – 3	**Clipboard outside of animal housing facility or in** [redacted]	**Dr.** [redacted] **,** [redacted]**,** [redacted]**, or** [redacted]**,** [redacted]**.**	(**X**)	( )

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

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4 MPLS VAHCS NOV 2013

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	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	Irradiation	Imaging	Other**
1	**Scheduled fluid access protocol**	(**X**)	( )	( )	( )	( )	( )	( )	( )
2	**Chair restraint**	( )	(**X**)	( )	( )	( )	( )	( )	( )
3	**Operant behavioral conditioning and testing**	( )	( )	( )	( )	(**X**)	( )	( )	( )
4	**MRI and CT structural brain scans**	( )	(**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.
►

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 ► **Scheduled fluid access protocol.**
Scheduled fluid procedure: During training sessions, the fluid intake of the animals is carefully controlled because fluid reward is used for the behavioral training. The animals get a substantial amount of water during the training sessions and this amount is supplemented to a minimum daily amount (20-30 ml/kg), if not enough fluid was taken in a given day. Moreover, during training periods the animal is given water ad libitum overnight every 7 days, with the following exception*.

*Some animals develop the habit of "tanking up" during this overnight ad libitum, and are able to carry this higher hydration level over into the next few days. This elevated hydration level reduces the animals' motivation to perform the task in which they have been trained; in general, this occurs during the first two days of the week thereby significantly prolonging the duration of the experiment. Therefore, in an attempt to achieve a more regular, stable performance in such animals, and after consultation with the veterinarian and documentation in the animal's medical record, we will reduce the overnight ad libitum to an amount approximately 1.25 – 3 times the average daily intake (25-60 mL/Kg.): about 200 – 600 mL ('restricted ad libitum'). The absolute minimum for day 7 during this restricted ad libitum period would be 25 mL/Kg. In these special cases, we will be especially diligent in monitoring the animals' hydration level through more frequent use of Urine Specific Gravity (USG) measurements; during the first week of implementation, attempts will be made to take this measurement daily and periodically thereafter until the end of the restricted ad libitum period. We will weigh the animals several times each week and monitor their food intake. Changes in appearance and behavior will also be considered in determining hydration status. The monitoring of hydration is complex and is based on multiple factors, not urine specific gravity alone. During this period, the veterinarian will be kept informed of any deviation of the various measures from the normal

values. Restricted ad libitum would be limited to three weeks out of the month; e.g. at least one day of true ad libitum water would occur per month.

When the animals are not in training, water is provided ad libitum. During periods of scheduled fluid procedure, the condition of the animals for possible dehydration is tested by measuring the urine specific gravity periodically (approximately once every week if fresh uncontaminated urine specimens can be obtained from the home cage) and body weight at least once a week. Hydration will be adjusted in an attempt to maintain urine specific gravity at a reading of approximately 1.040 or less.

Special Procedure 2 ► **Chair restraint.** The animal is seated comfortably in a primate chair. This is necessary for behavioral training and recording of brain cell activity using microelectrodes. The monkey is adapted to chair restraint using food reinforcement and by gradually increasing the duration of time spent each day in the primate chair. At first, monkeys are chaired for short durations (< 30 minutes), and given frequent fruit rewards as positive reinforcement. Typically within a week, monkeys sit quietly without struggling in the chair for 1-2 hours. The duration of chairing is extended to 3-6 hours during behavioral conditioning (below). .

Special Procedure 3 ► **Operant behavioral conditioning and testing.** The animal is trained to successfully perform various cognitive tasks using reward-based training. Both primate chair restraint and behavioral training will not produce pain in the animals, and will last 3 - 6 hours daily (5 days a week). Once monkeys are fully trained to perform the tasks, behavioral testing continues throughout microelectrode or electrode array recording of neural activity: The position of the head is fixed by bolting head restraint devices incorporated in the cranial implant to an external brace while microelectrode recordings are obtained. Microelectrodes are introduced into recording chambers and inserted directly through the into the brain to record the electrical activity of neurons during task performance. Linear electrode arrays are inserted through a guide tube that just punctures the dura. Neural recording will last 3 - 6 hours daily, 5 days a week. Recording of eye movements is performed using an infrared eye tracker, will not produce pain in the animals, and will last 3 - 6 hours daily throughout training.

Special Procedure 4 ► **MRI and CT structural brain scans.** The animal will be placed in a primate chair and brought to the laboratory where it will be anesthetized with ketamine (7.0 mg / kg) and xylazine (0.6 mg / kg). The monkey will be placed on a gurney and its head will be positioned within a MRI compatible plastic stereotaxic frame. The monkey will be covered with drapes and wheeled from [redacted] to [redacted] to the MRI facility or CT facility in [redacted]. The patient MRI/CT gurney will be covered with sheets and waterproof nylon covers, and the monkey placed on the sheets, and then the gurney will be extended into the bore of the MRI magnet or CT scanner. Scans typically take approximately 45 minutes to acquire. About half way through the scanning procedure (approximately 45 minutes after original dose), the depth of anesthesia will be examined and augmented with about half the original dose of ketamine. After the scan is complete, the monkey will be removed from the stereotaxic apparatus, wheeled back to [redacted], and the monkey recovered from anesthesia in a primate chair until it can maintain upright posture independently and is alert. The monkey will then be transported to its home cage.

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

Special Procedure 1 ► **Scheduled fluid access is necessary to sustain the reward value of liquid earned during performing behavioral tasks. Ad libitum access to water outside of the context of behavioral training nullifies the reward value of liquid earned by successfully performing the task. Therefore, for training to be possible, it is necessary that monkeys do not have ad libitum access to water outside of the context of behavioral training. On most days and in most cases, monkeys meet or exceed their minimum daily fluid requirements by working in the behavioral tasks. On days in which monkeys earn insufficient fluid, the balance of fluid necessary to meet their daily minimum requirement is given to them in their home cages after training. With careful monitoring, monkeys are maintained in a healthy condition under this scheduled fluid protocol.**

Monkeys working under scheduled fluid access generally work until they reach satiety and voluntarily stop working for additional fluid reward. Rhesus macaques in the wild frequently encounter conditions in which ad libitum access to water is not available. Dr. Robert Desimone, and colleagues (Desimone et al. 1992) provide the following on this point: "we contacted two primatologists who have studied primate behavior in the wild (Dr. Stuart Altman, University of Chicago, Department of Ecology and Evolution; and Dr. Donald

Lindburg, Center for Reproduction of Endangered Species, Zoological Society of San Diego). According to both of them, access to water once a day is not uncommon for wild monkeys, particularly during dry seasons; thus the paradigm is not outside the range of natural conditions. Dr. Stuart Altman, who has observed the drinking patterns of baboons (Altman and Altman, 1970), states: "at Amboseli, baboons occasionally go an entire day without water, when the nature of their day range precludes drinking." And further: "Dr. Donald Lindburg, who has studied the drinking patterns of rhesus monkeys (Lindburg, 1977), states: 'as conditions began to dry out and daily temperature to rise, the troops would travel distances of 1-2 miles on a daily basis to visit a water source' and expresses the opinion that 'a laboratory macaque living indoors at moderate air temperature could do quite well on access to water once or twice daily." In regards to adaptation to controlled water access, Toth and Gardiner (2000) state: " What is crucial is whether the animal learns to modify its patterns of ingestion to adjust to the overall availability of feeding and drinking opportunities across days. This situation is faced both by animals living in natural environments and by animals in laboratory restriction models." And these authors further conclude: "In summary, the restricted availability of water for intervals up to 24 h causes the sensation of thirst, but even chronic restriction schedules do not cause physiologic impairment assuming the animals are adapted to the restriction schedule and receive enough water to replenish daily losses." In regards to controlled water access paradigms, the Guidelines for Diet Control in Behavioral Studies, provided by the Animal Research Advisory Committee of the NIH, states: "Animals routinely adapt well to the paradigm and display no signs of distress."

REFERENCES

Altman S and Altman J (1970) Baboon Ecology: African Field Research. Chicago: University of Chicago Press.

Desimone R, Olson C, and Erikson R (1992) The controlled water access paradigm. ILAR news, 33:48-52, 1992. Silverman J, Suckov AM, and Murthy S (2000) The IACUC Handbook. CRC Press; Boca Raton.

Toth LA, Gardiner TW (2000) Food and water restriction protocols: physiological and behavioral considerations. Contemporary Topics in Laboratory Animal Science, 39(6): 9-17.

National Institutes of Health (2002). Guidelines for diet control in behavioral studies. Animal Research Advisory Committee.

Lindburg DG (1977) Feeding behaviour and diet of rhesus monkeys (Macaca mulatta) in a siwalik forest in northern India. Pp. 223-249 in Primate Ecology: Studies of Feeding and Ranging Behavior in Lemur, Monkeys, and Apes, T. H. Clutton-Brock, ed. New York: Academic Press.

Special Procedure 2 ► **Chair restraint is necessary for the safety of both monkeys and personnel during transport of monkeys between the housing facility and the laboratory, as well as behavioral testing and neural recording in the laboratory. This limits the potential that monkeys could bite or scratch personnel working with them or injure themselves. Chair restraint is necessary to position the monkey in front of the video display during behavioral testing, and during neural recording (in conjunction with head restraint) so that it is possible to position externally supported microelectrode recording matrices within implanted recording chambers.**

Special Procedure 3 ► **Operant conditioning and behavioral testing of monkeys is necessary to establish reliable and accurate performance of cognitive behavioral paradigms, and to relate neural signals to behavior. Neurophysiological recording is necessary to relate the electrical activity of individual neurons to cognitive information processing.**

Special Procedure 4 ► **Structural MRI and CT brain scans are necessary to visualize the anatomical locations of specific cortical and subcortical brain structures, in order to be able to surgically locate craniotomies and recording devices over the brain areas of interest. Additionally, the CT scan will allow us to determine the shape of the skull to determine the correct design of the chamber to allow us to hit subcortical target areas. The CT scan will also allow us to determine if the DBS probe is correctly placed following implantation before we start the stimulation experiment.**

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
1-4	**Dr.** [redacted]	**Dr.** [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	(**X**)		( )[a]	( )[b]
2	(**X**)		( )[a]	( )[b]
3	(**X**)		( )[a]	( )[b]
4	(**X**)		( )[a]	( )[b]

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					
4					

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

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

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

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

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	**During the scheduled water access protocol, monkeys will be weighed not less than once weekly, and their food intake will be recorded daily. In addition, their urine specific gravity will be monitored periodically (approximately once every week if fresh uncontaminated urine specimens can be obtained from the home cage)** Urine specific gravity measurements will also be taken several (~3) times prior to the onset of scheduled fluid procedure to provide a baseline level for the individual animal.	**If a period of reduced eating in response to the scheduled water access protocol occurs during which a monkey loses more than 20% of the body weight it maintained while healthy and working under the water deprivation protocol, we will make the training conditions easier by simplifying the task and making the earning of liquid rewards easier to increase fluid intake above the 20 ml/kg minimum. This should help to increase food intake. If we are not able to achieve a positive change in the fluid intake within a 2-week period, (meaning an increase in food intake and body weight), we will place the monkey on ad libitum water and consult a Staff Veterinarian concerning alternative training and or reward options before continuing behavioral training.**
2	**Monkeys will be monitored continuously during chair restraint while they are transported between the housing facility and the laboratory. Monkeys will be monitored approximately once every 20-30 minutes during chair restraint while undergoing automated behavioral training, and continuously during chair restraint while neural recording is being conducted.**	**Monkeys adapt well to chair restraint and sit in the chair for extended periods (3-6 hours) of head restraint calmly while remaining still during behavioral testing and neural recording. However, it could be difficult for individual monkeys to adapt to chair restraint. In this case, we will shorten the period of chair restraint and increase food rewards given during restraint until the monkey sits quietly and works consistently on the task. We will limit the period of behavioral testing and neural recording to the time that monkeys will sit quietly and work on the task with minimal or intermittent movement.**
3	**Monkeys will be monitored approximately once every 20-30 minutes during automated behavioral training, and continuously during neural recording and stimulation.**	**If a monkey is unable to achieve an acceptable level of performance at a given stage of training (typically above 80% correct), we will make the training conditions**

		easier by simplifying the task until this level of performance is achieved, before advancing training to the next stage. Often, if monkeys have difficulty in training simplifying the training stages and giving animals more time to master each stage before increasing task complexity is effective in improving performance and fluid intake. Although we have been successful in this effort thus far training more than 15 monkeys on various cognitive tasks, should it prove impossible to train a monkey on a future task, we will remove the monkey from the study. This has never occurred in our past experience and so is not anticipated. If a monkey cannot be used for behavioral testing in these experiments, we will make every effort to find another principle investigator and laboratory, either at the VA, the University of [redacted], or another US research institution, that could use the animal in a different experiment, with less demanding behavioral requirements, and arrange for transfer of that animal to that lab under a different protocol. If we are not successful in this effort over a period of 6 months, the animal will be euthanized.
4	**Monkeys will be visually monitored continuously during transport between [redacted] and [redacted], and continuously during the MRI (~30 minute scan time) and CT (~10 minute scan time) scanning procedure. If feasible to use a pulse oximeter instrument with the MRI or CT scanning equipment, we will monitor pulse and blood oxygenation level at the beginning, half way through, and at the end of the scanning procedure (approximately every 10-15 mins).**	**We will not carry out the scanning procedure in monkeys that exhibit contraindications for anesthesia, such as a chronic or serious medical condition and/or advice of the veterinary staff.**

ACORP APPENDIX 7
USE OF PATIENT CARE EQUIPMENT AND/OR AREAS
FOR ANIMAL STUDIES
VERSION 4 MPLS VAHCS NOV 2013

See ACORP App. 7 Instructions, for more detailed explanations of the information requested.

1. **Full Name(s) of Principal Investigator(s) ►** [redacted], Ph.D.

2. **Equipment to be Used.**

   a. Identify the equipment ► **Patient MRI instrument and CT scanner**

   b. Procedure(s) to be performed with this equipment ► **MRI brain scan and CT brain scan**

   c. Describe how contamination of the human patient care equipment will be prevented and how the equipment will be cleaned/sanitized before its subsequent use for human patients.
   ► **The MRI and CT instrument on which the animal lies is completely covered with appropriate material (sheets with nylon covers) so that the animal does not touch the instrument. In addition, all personnel involved in this procedure wear all appropriate BSL-2 personal protective equipment at all times during the procedure. The MRI and CT instrument on which the animal lies are disinfected after the animal is removed by wiping down patient gurney with CiDecon Plus wipes or comparable hospital disinfectant.**

3. **Human Patient Care Procedural Areas to be Used.**

   a. Location(s) ► **MRI:** [redacted]**, Room** [redacted] **and CT:** [redacted]**,** [redacted]

   b. Animal species to be studied or treated ► **Macaca mulatta**

   c. Number of individual animals to be studied or treated ► **8**

   d. Date(s) ► **The dates depend on the course of the experiment and the patient schedule for the MRI or CT facility.**

   e. Time(s) of day ► **MRI or CT will be done after hours, when no patient's MRI or CT are scheduled.**

   f. Procedure(s) to be performed on the animals in these areas ► **Acquisition of structural MRI and CT images of the brain.**

   g. Protection and cleaning of patient care room surfaces ► **The MRI and CT instruments on which the animal lies is completely covered with appropriate material (sheets with nylon covers) so that the animal does not touch the instrument at any time during the procedure. The MRI and CT instruments on which the animal lies is disinfected after the animal is removed by wiping down patient gurney with CiDecon Plus wipes or comparable hospital disinfectant.**

   h. Benefits to VA patients**.** Briefly describe how this use of the human patient care areas for research on animal subjects potentially benefits VA patients.
   ► **This research will benefit VA patients by elucidating the neural mechanisms of higher cognitive functions disrupted by several diseases affecting the veteran population, such as schizophrenia. The project involves studying the electrical activity of single neurons specific cortical and subcortical areas, and this critically depends on being able to localize the intended brain areas so that recording chambers can be accurately placed. The use of the patient MRI and CT facility makes this possible.**

   i. Necessity for use of human patient care areas. Explain why this work on animal subjects cannot be performed

within the animal facility or a research laboratory area.
► **No animal facility for structural MRI or CT scans for in monkeys exists at this VA.**

j. Animal transport. Describe how the animals will be transported back and forth between the animal housing area and the human patient care areas.
► The animals are anesthetized, and placed in a MRI and CT compatible stereotaxic apparatus in [redacted]. The monkey and stereotaxic apparatus are lifted onto a wheeled cart. The monkey and stereotaxic apparatus are completely covered with a surgical drape. The cart is then wheeled through the [redacted] and [redacted], to the MRI facility ([redacted], room [redacted]) or the CT facility ([redacted], room [redacted]). Special care is taken so that there is no contact with non-research personnel. The MRI is conducted [redacted], when no patients are present in the MR suite, CT suite, or adjoining areas. The distances between [redacted] (where the animals are housed) and the MRI and CT facilities in [redacted] are short, and the areas involved are normally deserted when the scans are done.

k. Preventing human patients and patient care personnel from being affected by the presence of the animals. Provide detailed descriptions of the measures to be taken to address noises and odors, allergens, and zoonotic pathogens associated with the animals.
► The monkey will be accompanied by Research Study Staff at all times during the scanning procedure and will be responsible for handling and monitoring the monkey at all times. Personnel that operate the MRI and CT scanning facilities will not be required to handle or have direct contact with the monkeys. The MRI and CT instruments on which the animal lies are completely covered with appropriate material (sheets with nylon covers) so that the animal does not touch the instrument. Any individual in the same room as the nonhuman primate would be fully garbed in BSL-2 level personal protective equipment. The individual(s) operating the scanner are normally located in a separate room and thus would not need this level of PPE. Because the MRI and CT are done [redacted], there are no patients or people in the area who would be disturbed by the procedure.

4. **Signatures.** Provide the signatures required on the signature pages (Item Z.7) of the main body of this ACORP.