The University of Mississippi Medical Center

Animal Activity Protocol

IACUC - Institutional Animal Care and Use Committee
Telephone [redacted] / Facsimile [redacted]
iacuc@umc.edu

To be completed by IACUC

Protocol Number: **1389B**	Date: **9/18/2019**	Classification: **D**

1. Principal Investigator

Name	[redacted]		
	☒PhD ☐ MD ☐ Other:		
Title	**Professor**		
Dept.	**Psychiatry & Human Behavior**		
Phone #	[redacted]	**Office Location**	[redacted]
email	[redacted]	**Emergency #**	[redacted]

Note: The emergency number should be a number at which the PI can be contacted on nights and weekends.

2. Other Personnel

All listed personnel must complete IACUC required training, including completion of Occupational Health forms and submit a Training Requirements Registration form prior to working with animals and receiving access into the Center for Comparative Research (CCR).

You may authorize personnel to submit modifications to this protocol by checking the box for signing privileges.

Name	Title	Ext/Cell	Email	Signing Privileges
[redacted]	Professor	[redacted]	[redacted]	☒
[redacted]	Research Operations Specialist	[redacted]	[redacted]	☒
[redacted]	Associate professor	[redacted]	[redacted]	☐
[redacted]	Assistant professor	[redacted]	[redacted]	☐
[redacted]	Instructor	[redacted]	[redacted]	☐
[redacted]	Post-doctoral fellow	[redacted]	[redacted]	☐
[redacted]	Post-doctoral fellow	[redacted]	[redacted]	☐
[redacted]	Researcher II	[redacted]	[redacted]	☐

Name	Title	Ext/Cell	Email	Signing Privileges
[redacted]	RA II		[redacted]	Added 5/11/21
[redacted]	RA II		[redacted]	added 5/13/21
[redacted]	INBRE student			added 5/27/21
[redacted]	Researcher II		[redacted]	added 6/8/21
[redacted]	Researcher II		[redacted]	Added 6/14/21
[redacted]				Added (08/14/21)
[redacted]	Med Student		[redacted]	Added 9/3/21
[redacted]	Researcher II		[redacted]	Added 11/4/21
[redacted]	Graduate Student		[redacted]	Added 2/1/22
[redacted]	Researcher II	[redacted]	[redacted]	Added 4/21/2022
[redacted]	Post- Doc Fellow	[redacted]	[redacted]	added 5/18/22
[redacted]	Researcher II		[redacted]	added 8/18/22

[redacted]	Graduate Student (PIN/Psychiatry)	[redacted]	[redacted]	☐
[redacted]	Head of UMMC Behavioral Core	[redacted]	[redacted]	☐
[redacted]	Lab Manager – [redacted]	[redacted]	[redacted]	☐
[redacted]	Researcher III – [redacted]	[redacted]	[redacted]	☐
[redacted]	Researcher II – [redacted]	[redacted]	[redacted]	☐
[redacted]	SURE Student	--	[redacted]	☐
[redacted]	SURE Student	--	[redacted]	☐
[redacted]	SURE Student	--	[redacted]	☐
[redacted]	MSRP Student	--	[redacted]	☐

(Insert additional lines as needed)

3. Project Title:

Behavioral Pharmacology Studies in monkeys: Sedatives, analgesics, and stimulants.

4. Proposal is **3 year Full Submission Renewal (must attach Appendix K)**

5a. Outside Contracts

Will any components of this study involve live animals maintained at another institution?

☒ No

☐ Yes (if yes, provide information on the level of involvement)

5b. Animal Behavior Core

Will this study use the Animal Behavior Core (ABC)?

☒ No

☐ Yes – Requires review and approval by ABC Director. See Appendix L.

6. Funding Source

☒ Extramural/Intramural Funding

Title	Anxiolytic and abuse-related effects of BZ ligands		
PI	[redacted]		
Funding Agency	NIH/NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R01 DA011792
Covered Dates	6/1/1998-12/31/2019		

Title	Tolerance and Physical Dependence after Chronic Benzodiazepine Treatment
PI	[redacted]

Funding Agency	NIH/NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R01 DA043204
Covered Dates	7/1/17-6/30/22		

Title	Opioid and benzodiazepine co-abuse in nonhuman primates		
PI	[redacted]		
Funding Agency	Alkermes		
Status	☐ Submitted ☒Funded	Grant Number	PW014-2018
Covered Dates	01/01/2019 – 12/31/2020		

Title	EEG Telemetry in Monkeys: Potential Markers of Benzodiazepine Action		
PI	[redacted]		
Funding Agency	NIH/NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R21 DA046778
Covered Dates	06/01/2019 – 05/31/2021		

(Copy and paste table if project is funded by multiple grants.)

☐ Department – List Department:

☐ Other (*Example: Divisional funds which you have control over, start-up funds*)
Explain:

7. Dates of Study

Anticipated start date of study: 9/1/2019

All investigators must adhere to a federally mandated three-year cycle of full protocol review, even if a funding period exceeds three years in duration.

8. Source of Animals

Will any animals be obtained from non-commercial sources? ☒No ☐Yes
If Yes, list:

Note: Animals from non-commercial sources must have their health status evaluated by a CCR veterinarian prior to their arrival at UMMC. This question does not relate to the acquisition of animals from other UMMC investigators. If animals are transferred from a UMMC source, an Animal Transfer Form must be completed and approved for each transfer.

9. Animal Requirements

For **New** submissions complete **Table A.**
For **3 Year FSR** submissions complete **Table B**.

Animal numbers MUST be calculated for a period not to exceed three (3) years from the start of the study.

A. New:

Species	Strain/stock	Sex	Source	Total for 3 years	Average daily census

(Insert additional lines as needed)

Note: If using nonhuman primates, complete Appendix A.

B. 3 Year FSR: **For a 3-year renewal, number of animals needed to complete the studies in this protocol. This must include the number of animals to be received plus the number of animals <u>currently on campus</u> to be carried over from the previous version of this protocol.**

Example: You need 100 animals to complete your study and you have 20 animals currently in house to carry over to this is protocol.

Total Needed for 3 years		*Total Carried Over*		*Total Requested*
100	-	20	=	80

You will be approved for 100 animals to complete the study (number to be justified in question #17) of which you already have 20, so you will have 80 animals available to order.

Species	Strain/stock	Sex	Source	Total Needed For 3 years	Total Carried Over	Total Requested (Needed – Carried Over)	Average daily census
NHP	**Rhesus Macaque**	**M/F**	**National Primate Centers (e.g., CNPRC, Yerkes, etc.).**	**50**	**29**	**21**	**50**

(Insert additional lines as needed)

Note: The number of animals available for ordering will be the difference between total animals needed minus carryover animals.

C. List any unusual phenotypes or abnormalities associated with the animals (including sublines) listed above (i.e., prone to diarrhea, decreased appetite, patchy hair loss, increased sensitivity to pain, slow wound healing, etc.).

10. Breeding program

Will animals be involved in a breeding program at UMMC or will time-pregnant animals be used?

☒ No

☐ Yes (if yes, provide information in Appendix B)

11. Potential Hazards

		Yes	No	Pending
A	**Chemical toxins used in animals?**	☐	☒	
	Reviewed by Environmental Health & Safety?	☐	☐	☐
B	**Radioisotopes used in animals?**	☐	☒	
	Reviewed by Radiation Safety?	☐	☐	☐
C	**Use of laser, CT, x-ray, or fluoroscopy?**	☐	☒	
	Reviewed by Radiation Safety?	☐	☐	☐
D	**Biohazards used in animals?**	☐	☒	
	Reviewed by Institutional Biohazard Committee?	☐	☐	☐
E	**Human cells used in animals?**	☐	☒	
	Reviewed by Institutional Biohazard Committee?	☐	☐	☐

If YES, provide specific details of specialized animal husbandry, care, cleaning, or decontamination procedures**, especially identifying responsible parties**.

Although biohazards are not used in the animals, the monkeys themselves represent a biohazard risk in that they carry macacine herpesvirus 1, or Herpes B virus. In order to reduce the risk of exposure, proper PPE is worn at all times in monkey areas. This includes a lab coat, face mask, face shield, gloves, pants and closed-toed shoes. In the event an exposure does occur, a biohazard protocol is in place such that the person must scrub the affected area with a betadine pad for 15 minutes (for scratches, cuts, bites, etc.) of flush the affected area for 15 minutes (e.g., eye & mouth exposures). Following this, blood is taken from both the exposed person and the monkey and sent off to test for the Herpes B virus, with a follow-up blood test occurring 2 weeks later. **The lab has obtained IBC approval for these procedures, and signed informed consent forms for all lab members are on file in the IBC office as well as within the lab.**

12a. Animal Husbandry

	Standard	Nonstandard
Feeding	☐	☒
Watering	☒	☐

Caging	☒	☒
Room/Environment	☒	☐
Altered light cycle	☒	☐

Note: Provide complete explanation and justification for any **nonstandard animal husbandry** (e.g. metabolic caging, restraint chairs, transport devices, singly housed animals, altered light cycle). Protocols listing non-standard husbandry must provide complete details of the cleaning and sanitation, **especially identifying responsible parties**:

Feeding: Monkeys will be fed a prescribed amount of monkey chow by lab personnel listed on the protocol Monday-Friday (not including holidays). CCR personnel feed the monkeys the same amount of monkey chow on weekends and holidays. Amount of chow for each monkey will be determined in consultation with veterinarians to be that which maintains healthy weights in rhesus monkeys and also allows for criteria-level performance on behavioral tasks. For monkeys on studies that involve food reinforcement or drug/food choice, the amount of food consumed within-session will be subtracted from their daily food ration that is fed post-session. This allows for the maintenance of healthy body weights regardless of how much food is consumed within-session. Physical exams (including determination of weight and body temperature, hands-on assessment of body condition and somatometric measurements to prevent major fluctuations in weight, examination of eyes/ears/teeth/tongue/lips for lesions associated with the Herpes B virus, etc.; hands-on palpation of abdomen for presence of masses, examination of incision sites, catheter exit sites, and pretreatment drug injection sites for adverse consequences) will be conducted every 1-2 months by listed personnel. Monkeys will be lightly sedated with ketamine (~10 mg/kg, i.m. or i.v.), or other sedative-anesthetic as administered by CCR staff, for these exams.

Caging: Some studies will be conducted in the monkey's individual living quarters. Monkeys will self-administer intravenous (i.v.) infusions of drugs/compounds. The i.v. catheter exits the monkey's back or is attached to a subcutaneous vascular access port and is threaded through a tether attached to a swivel which can be inserted into a self-administration panel on the side of the home cage. Monkeys cannot be pair-housed while on home-cage self-administration studies due to the risk of damage to the tether, catheter, etc. (see Appendix A for more information on pair-housing exemptions), and the self-administration panel inserted into one side of the cage prevents visual contact with other cages/monkeys on that side of the monkey. The monkeys do still have visual, auditory, and olfactory contact with others in the room via the front of their cage unit as well as the side opposite the self-administration panel.

12b. Singly Housed Animals

Will animals be singly housed?

☐ No

☒ Yes – Please provide justification for single housing. **NOTE: If using non-human primates you must complete Appendix A.**

Appendix A attached for singly-housed non-human primates.

13. Housing

Will animals be housed outside of the LAF for greater than 12 hours?

☒ No

☐ Yes Where?

Note: If yes, provide complete explanation and justification for any **decentralized animal housing.**

14. Objectives in lay terminology

In **non-technical**/lay terminology, what is the **objective of the experiments** proposed in this Animal Activity Protocol? (i.e. ***Response should be written in non-scientific language, as though explaining the study to a high school student***.)

- In non-technical**/lay terminology**, what is the objective of the experiments proposed in this Animal Activity Protocol?
- Why are the experiments proposed?
- What knowledge do you hope to achieve?
- What is the potential relevance (e.g. benefits) of experimental findings to human or animal health, advancement of knowledge, and/or the good of society?

Generally, **single sentence explanations** for these types of questions will suffice.

Benzodiazepines (BZs) are widely prescribed as anxiety-reducing drugs, sleep aids, anticonvulsants, and muscle relaxants. Although BZs are considered to be among the safest psychoactive drugs in contemporary medicine, their clinical utility is constrained by unwanted side effects, including addiction and physical dependence, as well as balance issues and daytime drowsiness. The "big picture" goal of our research is to understand how certain brain molecules, referred to as "GABA-A receptors" are involved in both the medically useful and detrimental effects of these important drugs.

Overall, our research's over-arching goals are as follows:

1. For approximately 20 years, we have investigated the role of GABA-A receptors in the anxiety-reducing ("anxiolytic"), sleep-inducing, sedative, motor-impairing, cognition-impairing/enhancing, and abuse-related effects of BZs and related drugs/compounds in non-human primates. BZ-type drugs that vary in how they act at GABA-A receptors are used as probes for assessing mechanisms underlying the relevant behavioral effects of investigational drugs in monkeys.

2. BZ-type drugs are remarkably effective in treating anxiety disorders, but a major problem with these drugs are side effects, including significant abuse liability. In fact, the abuse of BZs appears to be on the rise in the US, making the need for improving the side effect profile of these drugs a clear priority. One of our research programs examines a novel approach to reducing BZ abuse: we are exploring novel combination products. One study primarily focuses on the use of a standard BZ and another type of drug referred to as a "neuroactive steroid" that in preliminary studies showed enhanced anxiety-reducing effects and virtually no enhancement of abuse potential.

3. As one of the main uses for BZs in humans is for the treatment of sleep disorders, a new research program in our laboratory has been using state-of-the-art implantable telemetry technology to conduct polysomnography to evaluate the effects of BZs on sleep-wake cycles in monkeys. Polysomnography is the "gold standard" test used in the study of sleep and as a diagnostic tool in sleep medicine. Our totally implantable telemetry system allows us to precisely study sleep in rhesus macaques, a species with sleep patterns very similar to humans. With this program, we hope to explore fundamental mechanisms of action underlying drug-induced sleep alterations, with the hope of discovering novel medications for treating insomnia and other sleep disorders not involving widespread abuse potential.

5. An unexpected gain from BZs having memory impairments was the discovery that some compounds that are related to them chemically actually act in the opposite direction. These compounds are referred to as “inverse agonists,” and just as BZs impair memory, the inverse agonists may, in fact, improve memory. Our primary approach to studying the role of GABA-A receptor subtypes in drug-induced cognitive effects is to evaluate the extent to which deficits and/or enhancements occur in tasks that make up the monkey Cambridge Neuropsychological Test Automated Battery, or CANTAB. the CANTAB is a touch-screen device commonly used in human patients to assess learning and memory. Thus, the use of CANTAB greatly facilitates translation of findings from animal models to humans (and vice versa).

15. Rationale

A. What is the rationale for using animals rather than using non-animal models?

The work described in this protocol cannot be conducted using solely tissue samples or biological materials in vitro because a primary goal of the research is to determine how drugs/compounds alter and control behavior in models predictive of effects in humans. Computer modeling does not provide meaningful information for this type of research, since modeling depends largely on a priori information that is not available without first conducting the types of studies described herein.

B. What is the rationale for using the particular animal species and/or strain noted in Item 9?

Rhesus macaques are ideally suited for preclinical research on drug and alcohol addiction, and for many questions in addiction research are considered to be the “gold standard” species, particularly by federal regulatory agencies such as the FDA and DEA. This species has been used in behavioral pharmacology research for over 40 years and has provided valid and reliable models of multiple aspects of substance use disorders. Because of the extensive use of rhesus macaques in neuroscience research, there is a large body of scientific information which provides indispensable comparative information for proper interpretation of our research. Finally, our new initiatives in sleep and sex differences are empowered by the observation that (1) sleep architecture of rhesus monkeys is very similar to that found in a human, and (2) rhesus monkeys have menstrual cycles that essentially parallel exactly that observed in humans.

16. Brief Outline

Provide a general description of the animal procedures included in the experimental design.

- **Briefly outline** the proposed animal manipulations and provide a time-line of events.
- **Note that specific details about methods and procedures will be required in the appropriate appendix (see list below)**
- **Complete** only those **appendices that apply** to the animal manipulations in your experimental design.
- If possible, flow charts and/or time lines should be included to clarify the timing of procedures which are to be performed.

Verbatim descriptions from a grant submission are not acceptable and will not be reviewed.

Subjects in all studies are male and female rhesus macaques (Macaca Mulatta) obtained from commercial sources and/or national primate research centers (e.g., Tulane, NEPRC, etc.). Our studies involve experiments in home cages or training of animals to sit in restraint chairs in order to conduct behavioral/cognitive tasks. Blood draws and vaginal swabs may be conducted in awake animals after a period of training. Studies also may involve placement of chronic indwelling intravenous catheters and/or implantation of monitoring devices or EEG leads.

Benzodiazepines and related drugs are some of the safest medications known to psychiatric medicine. This has been proven to be the case in non-human animal research as well, and has been our experience: in 20+ years of research with these drugs and sources of drug/compounding ranging from academic laboratories to large pharmaceutical companies, we have never experienced a drug overdose resulting in any long-lasting adverse effect to the animal. Nevertheless we do appreciate that secondary harm may result, i.e., aspiration in a deeply-sedated animal. To reduce this risk, we typically limit the amount of exposure to the drug or experimental compound to the minimum required to reliably answer the particular scientific question. In drug self-administration studies, we do not allow unlimited access, typically programming a cut-off to a safe number of injections per unit of time. Several equipment safeguards are in place to reduce or eliminate risk of exposure due to equipment issues. The various types of experiments an animal may be involved in are listed below – for further detail, see appendix B (behavioral testing). Timeline estimates for all studies are based on past experience conducting behavioral research with monkeys – single-subject designs can often mean that training and testing periods are extended due to stability criteria imposed on the data.

1. Operant drug self-administration: These studies are conducted in the home cage. Monkeys are implanted with catheters one of 8 available veins (internal jugular, external jugular, brachial, femoral) using aseptic surgical techniques. To protect catheters, the animals are fitted with jackets (Lomir, inc.) and tethers mounted to a swivel. This swivel is inserted into a self-administration panel that attaches to the side wall of the home cage. Experiments involve training the monkey to press a lever in order to receive an i.v. infusion of drug – the number of lever presses required typically is determined by the type of study conducted. These studies allow us to determine the reinforcing effects, or abuse potential, of drugs and novel compounds. [Timeline estimates: Habituation 2-4 months, Training 2-6 months, Testing 6-18 months]

2. Operant behavior maintained by different stimuli: These studies are conducted in experimental cubicles with monkeys sitting in restraint chairs (Crist instruments, inc.; Primate products style- custom manufactured). As with #1, all animals are implanted with catheters, except that no tether is necessary. The monkey is either fitted with a jacket containing a Velcro-lined pocket in which the closed-off (with obturator or tie) catheter can be placed when not in use, or a subcutaneous vascular access port (Norfolk Med/Access Technologies) can be surgically implanted in the monkey's back at the catheter exit site which maintains the catheter completely subcutaneously and does not require use of a jacket. Monkeys press levers for food pellets or drug injections, depending on the study. For some studies (*conflict procedure*), an occasional lever press may result in very mild electric shock, which suppresses behavior. Drugs that are anxiety-reducing agents reverse this suppression, providing us with a model of anxiolysis. [Timeline estimates: Habituation 2-4 months, Training 4-12 months, Testing 2-12 months]

3. Observation procedures: Monkeys are outfitted in the home cage as described in #1, but levers are not made available. Drugs and/or novel compounds are administered through the catheter and trained observers measure species-typical ("normal") behavior, as well as characteristic drug-related behavior, such as sedation. Experimenters occasionally interact with the monkeys in order to conduct simple cognitive tasks or measure motor coordination. [Timeline estimates: Habituation 2-4 months, Training 12-24 months, Testing 6-12 months]

4. EEG studies: After recovering from surgery to implant EEG leads (see appendix C for more information), the effects of sedative, stimulant or analgesic drugs on daytime EEG and on sleep architecture will be investigated by giving animals subcutaneous, intravenous or intramuscular administration of drugs in the home-cage. For intravenous drug administration studies, animals are surgically prepared with a chronic indwelling venous catheter as with #1 and #2. Prior to administration of drugs, the telemetry implant is turned on to record EEG/EMG/EOG readings in a computer located outside of the room. In some studies, animals will be subjected to the multiple sleep latency test (MSLT), which allow us to determine the animal's sleepiness under baseline conditions or in response to different drug treatments. During MSLT sessions (total of 5 sessions distributed throughout the day; session lengths combined not to exceed 3.5 hours per day), animals are positioned in a primate chair (Crist Instruments, Inc.; Primate Products style-custom manufactured) and placed in a dark, ventilated, sound-attenuating experimental chamber. MSLT experiments involve placing the animals in the chamber at 2-hour intervals: 8 AM, 10 AM, 12 PM (noon), 2 PM, and 4 PM, during which sessions sleep onset will be determined as the time to the first 30-second epoch scorable as sleep. If no sleep is observed, then sleep latency will be designated as 20 minutes. Thus, for each trial, animals will be in the chair/chamber for no longer than 40min. Between naps, animals will be returned to their home-cages. MSLT sessions will be conducted no more frequently than 3 days per week. For all daytime and nighttime EEG studies, animals will receive approximately 6 injections/day at different times of the day, no more than 5x/week, and daytime EEG changes or full-night sleep scoring will be performed [Timeline estimates: Habituation= 2-4 months; Testing= 6-12 months].

5. Activity-based sleep parameters: To examine home-cage daytime activity and sleep parameters, we will use non-invasive Actiwatch monitors. Actiwatches may be placed in a protective case attached to a commercially-available nonhuman primate collar, or inserted into the back or side of the monkey's jacket. Placing these actiwatches inside of jackets may take place while the monkey is sedated, or while awake and secured in a restraint chair (see appendix G for details). Actiwatch monitors can continuously read activity and sleep data for 45-180 days, depending on the behavioral measure. Some subjects will wear the monitor continuously. Actiwatch monitoring may be done while the NHP is completing another study, such as CANTAB, sleep studies, EEG, or self-administration, and therefore does not have its own timeline(s).

6. Intravenous drug self-administration in the chair: A chronic intravenous catheter is implanted into the monkey, and the distal end is routed under the skin to a vascular access port located in the center of the lower back (see appendix C for more details). For the duration of self-administration sessions (not to exceed 3 hours per day), animals are positioned in a primate restraint chair (see appendix G. for more information) and placed in a ventilated, sound-attenuating experimental chamber. Once the monkey is inside the chamber, the catheter access port area is prepared under aseptic conditions (hair is removed using clippers, site is

prepared with 3 alternating applications of 70% alcohol and povidone/iodine prep solution), and a Huber needle (e.g., Access Technologies) is inserted into the access port. The tubing attached to the Huber needle is connected to a syringe pump located outside of the chamber containing the drug solution. For animals that do not have ports, the catheter will be kept in a pocket inside of the jacket (either tied off or with an obturator in it). Once in the chamber, the jackets of non-port monkeys will be opened and a sterile connecting pin will be used to attach the catheter to the syringe pump. Monkeys will be trained to press a lever in order to receive an infusion of a drug, with the number of lever presses required being determined by the type of study being conducted. These studies allow us to determine the reinforcing effects of drugs and novel compounds. Self-administration sessions will be conducted no more frequently than 5 days per week. [Timeline estimates: Habituation 2-4 months, Training 2-6 months, Testing 6-12 months]

7. Computer touchscreen-based cognitive tasks (CANTAB): As in #2, monkeys will be trained to sit in restraint chairs, implanted with chronic indwelling catheters, and placed in a quiet cubicle with a computer touchscreen. The monkeys are trained on tasks of spatial working memory, reference memory, set shifting, attention, basic stimulus-response relationships, delayed reinforcement assessment, and other cognitive tasks. These procedures allow us to assess both cognitive enhancing and attenuating effects of drugs and/or novel compounds. [Timeline estimates: Habituation 2-4 months, Training 12-24 months, Testing 6-12 months]

8. Tail dip procedure: This procedure is widely used as a test of analgesia in rhesus monkeys, and includes placing the lower portion of an animal's tail into heated water (temperature range: 40-52.5 degrees Celsius, plus or minus one degree). Analgesia is operationalized in this procedure as an increase in the latency to withdraw the tail from the heated water. In the absence of analgesics, the latency is shorter, and this latency is lengthened by analgesics (e.g., prescription opioids) in a dose-dependent manner. For our tests, monkeys are trained to sit in restraint chairs, and the latency to remove the tail completely from the water, or a maximum of 20-s, will be measured. We use a 20-s cutoff to limit exposure of the tail to heated water as a conservative protective measure. Notably, the tail-dip is an escapable situation. Subjects can easily withdraw their tail from the water at any time. [Timeline estimates: Habituation 2-4 months, Training 12-24 months, Testing 12-36 months].

Appendix A	**Environmental Enhancement/Enrichment**
Appendix B	**Breeding Programs**
Appendix C	**Surgery & Management of Surgical Pain & Distress**
Appendix D	**Collection of Biological Samples**
Appendix E	**Antibody Production**
Appendix F	**Administration of Drugs/Test Compounds**
Appendix G	**Prolonged Physical Restraint**
Appendix H	**Multiple Survival Surgical Procedures**
Appendix I	**Food and /or Fluid Restriction**
Appendix J	**Animal Pain and/or Distress**
Appendix K	**Progress Report**
Appendix L	**Behavior Testing and Training**

17. Justification of animal number

Explain and **justify** how the number of animals requested was determined. (Flow diagrams/tables to define animal use are encouraged. **Statistical support should be included.** This number should support the request made in the *Total for 3 years* column in #9 and be consistent with the outline in #16).

The number of monkeys to use in in-vivo pharmacology studies is a decision that involves a trade-off between using large numbers of animals and assuring the reliability of data collected using small numbers of animals. The proposed studies in this application are designed to increase the reliability of data from small numbers of monkeys by using, whenever possible, a within-subjects experimental design. This design, in which each animal serves as its own control, permits scientifically meaningful results to be obtained with fewer animals than would be required with other types of designs (e.g., an exclusively between-subjects approach). For some studies, we also require a between-subjects experimental design.

For the current protocol, we propose to use sample sizes in each study and/or group of N=4-6, which historically has been sufficient to draw meaningful conclusions without excessive use of our valuable monkey resource. These numbers are also confirmed with power analyses on a case-by-case basis, using G*power 3.0.10 software (effect size = 0.8, alpha = 0.05, two-tailed). We have used these within- and between-subjects designs since the inception of our first NIH grant in 1998, and this approach has a long and extremely rich history in behavioral pharmacology research dating back to the 1960's. Based on statistical power assessments obtained from analyses of previous data sets, 4-6 monkeys are sufficient to draw reliable conclusions from experiments of the type proposed here.

18. Location & transportation

A. Indicate room(s) where animal procedures will be conducted.

Room Number	Procedures performed
[redacted]	Home-cage behavioral tasks (self-admin, chairing, etc.).
[redacted]	Cognitive, behavioral and sleep tasks for chaired NHPs (CANTAB, tail dip, MSLT etc.).
[redacted]	Choice self-administration studies
[redacted]	CCR Surgical suites

(Insert additional lines as needed)

B. Studies involving animal transportation to locations other than the housing area must identify the animal transport device, the nature of the shrouds used to cover the transport device, and describe the route of transport. **Include transport within the LAF (e.g. IVIS, surgery room).**

Monkeys will be transferred from housing areas in the [redacted] (basement) to the nearby surgical suite. A cart will be used to transport the anesthetized monkey through the hall. For studies involving restraint chairs, the monkeys will be placed in their chairs in the colony room in the basement of the [redacted] building, and then transported to experimental chambers in room [redacted]. The chairs have large casters with brakes, along with handles, which facilitate pushing the chair safely.

19. Euthanasia

A. At what point in the proposed experiments will animals normally be euthanized, (experimental end-points)? Or at what point will any individual animal be euthanized?

Euthanasia is not a part of the proposed experiments and will be performed only as necessary (e.g., due to terminal illness).

B. What humane endpoints or criteria will be used to determine if an animal is to be euthanized prior to, rather than at, the anticipated end-point of an experiment? Note: Contact LAF, ext. [redacted], for recommendations on the assessment criteria.

Benzodiazepines and the related compounds described in this proposal are non-toxic by pharmacological/pharmaceutical standards and are well known to be some of the safest drugs known to clinical medicine. In this regard, doses of benzodiazepines > 1000-fold the effective doses in tests of anxiolysis and sedation do not result in death in laboratory animals (in fact, LD50 doses cannot be calculated for these drugs). Due to this safety profile, humane endpoints for euthanasia are not required for administration of these drugs or the related compounds.
The studies in this protocol all require chronic intravenous catheters. We have considerable experience (>20 years) in the implantation and maintenance of these catheters, and complications due to catheter implant are relatively rare. The complications usually involve infections or thrombosis-induced clogging of the catheter. Regarding possible infections, the site is checked (if not obscured by jacket) visually daily, by lab staff. For self-administration monkeys, catheter exit sites are inspected at minimum once every two weeks when the animal is sedated for the changing of the home cage, or for chaired monkeys, exit sites are checked on any day that chairing/experimental sessions occur. Any evidence of swelling, exudates, or redness as well as any elevations from normal body temperature will be reported to CCR veterinary staff for assessment and treatment, if necessary.

All of the PI's staff members are trained that rapid detection of infections will negate the unlikely chance of sepsis development. However, if sepsis were to occur, the disposition of the animal would be assessed by the CCR veterinary staff for weight loss, loss of appetite, and blood biomarkers of infection – euthanasia will be considered, in consultation with the PI, if sepsis is deemed untreatable.

Clogged catheters due to thrombogenic events are typically detected prior to an experimental session, when the catheter is flushed to assess patency. Clogged catheters are replaced with a new catheter, if possible. Our primary preventative measure is frequent flushing with sterile saline containing heparin, as described above. Clot formation resulting in cardiac dysfunction can occur, and is usually detected as a change in weight and appetite, sometimes accompanied by swelling in the extremities. These events will be reported to the CCR veterinary team for cardiac assessment and the decision to euthanize is made in consultation with the PI.

C. Will natural death (or death due to manipulations) be used as an endpoint?

☒No ☐Yes – if "Yes", explain and justify.

20. Euthanasia Procedures

What procedures will be used to euthanize the animals? Note: Secondary methods are required to ensure death. (Consult the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition for appropriate methods of euthanasia or contact the LAF.)

Euthanasia is performed by veterinarians in the CCR. Monkeys are first anesthetized with ketamine (10-20 mg/kg) then administered a commercial euthanasia solution, such as pentobarbital (e.g., Fatal Plus, Vortech Pharmaceuticals). Generally a dose of pentobarbital is given that exceeds 50-100 mg/kg, i.v., and the veterinarian confirms euthanasia by ausculting for a loss of heartbeat, checking peripheral reflexes, etc. Necropsies are typically performed post-euthanasia by veterinarians. In addition to providing information about the health of the animal prior to euthanasia and allowing for tissue collection, this acts as a secondary form of euthanasia in ensuring the animal is deceased.

Assurances

1. Have all personnel received a medical evaluation from UMMC Student/Employee Health and updated Occupational Health Information annually?

   ☐No ☒Yes

2. Have all personnel listed on this protocol been informed and understand their role in the experiments?

   ☐No ☒Yes

3. Review of the available resources and previous experiments have determined that the proposed activity is not unnecessarily duplicative of previously reported activities.

   ☐No ☒Yes

USDA Policy #12, "Consideration of Alternative to Painful/Distressful Procedures": states the following: ***The Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions, and replacements.***

List each potentially painful or distressing procedure included in these protocol:

Intramuscular and subcutaneous injections	Conflict Procedure
Surgical implantation of i.v. catheter	Blood draws
Tail dip procedure	EEG lead implantation
Vaginal swabs (female NHPs)	

To comply with Policy #12, investigators are required to conduct literature searches using **two different search engines (see below)** addressing each of the procedures listed above. Specific procedures listed may be utilized as key terms.

Additional assistance may be obtained by contacting the Rowland Medical Library reference desk at ext. [redacted]. See IACUC Guidance on Minimizing Pain and Distress in Animals and Searching for Alternatives.

Helpful Databases

(Please note: PubMed and Medline are the same and cannot both be used.)

☒Medline/PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
☐Toxnet (http://toxnet.nlm.nih.gov)
☐AWIC (http://awic.nal.usda.gov)
☐Agricola (http://agricola.nal.usda.gov)
☒Scopus (http://www.scopus.com/home.url)
☐Other ()

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	Reduction in numbers of animals used	Refinement to minimize pain or distress	Lack of unnecessary duplication
PubMed	7/1/19	ALL	Conflict Procedure	Animal model + rhesus monkey + conflict	☐	☒	☒	☒
PubMed	7/1/19	ALL	Conflict Procedure	Conflict + rhesus monkey + alternative	☒	☒	☒	☒
PubMed	7/1/19	ALL	Conflict Procedure	Conflict procedure + Rhesus monkey	☐	☐	☒	☒
PubMed	7/1/19	ALL	I.V. Self-administration	Animal model + rhesus monkey + self-administration	☐	☒	☒	☒
PubMed	7/1/19	ALL	I.V. Self-administration	Alternative + rhesus monkey + self-administration	☒	☒	☒	☒
PubMed	7/1/19	ALL	I.V. Self-administration	Rhesus monkey + self-administration + drug	☒	☒	☒	☒
PubMed	7/1/19	ALL	Surgery (catheter)	Alternative + rhesus monkey + surgery + catheter	☐	☒	☒	☒
PubMed	7/1/19	ALL	Surgery (catheter)	Animal model + rhesus monkey + surgery+ catheter	☐	☒	☒	☒
PubMed	7/1/19	ALL	Surgery (catheter)	Rhesus monkey + surgery + catheter + refinement	☐	☒	☒	☒

PubMed	7/1/19	ALL	I.M. injection	Animal model + rhesus monkey + intramuscular injection	☐	☒	☒	☒
PubMed	7/1/19	ALL	I.M. injection	Alternative + rhesus monkey + intramuscular injection	☐	☐	☒	☒
PubMed	7/1/19	ALL	Blood Draws	Alternative + rhesus monkey + blood draw	☐	☒	☒	☒
PubMed	7/1/19	ALL	Blood Draws	Animal model + rhesus monkey + blood draw	☐	☒	☒	☒
PubMed	7/1/19	ALL	Tail dip procedure	Animal model + rhesus monkey + tail dip	☐	☒	☒	☒
PubMed	7/1/19	ALL	Tail dip procedure	Alternative + Rhesus monkey + tail dip	☐	☐	☒	☒
PubMed	7/1/19	ALL	Tail dip procedure	Rhesus monkey + antinociception	☒	☒	☒	☒
PubMed	7/1/19	ALL	Vaginal swabs	Animal model + rhesus monkey + menstruation	☒	☒	☒	☒
PubMed	7/1/19	ALL	Vaginal swabs	rhesus monkey + vaginal swab	☐	☐	☒	☒
PubMed	7/1/19	ALL	Vaginal swabs	Alternative + rhesus monkey + vaginal swab	☒	☒	☒	☒
PubMed	7/1/19	ALL	Sleep Architecture	Sleep architecture + rhesus monkeys	☐	☒	☒	☒
PubMed	7/1/19	ALL	Sleep Architecture	Alternative + sleep architecture + rhesus monkeys	☒	☒	☒	☒
PubMed	7/1/19	ALL	Telemetry/Sleep	Telemetry + sleep + rhesus monkeys	☐	☒	☒	☒
PubMed	7/1/19	ALL	Telemetry/sleep	Alternative + telemetry + sleep + rhesus monkeys	☒	☒	☒	☒
PubMed	7/1/19	ALL	EEG/telemetry Surgery	Alternative + rhesus monkeys + telemetry + surgery	☒	☒	☒	☒
PubMed	7/1/19	ALL	EEG /telemetry surgery	Rhesus monkeys + telemetry + surgery	☐	☒	☒	☒
PubMed	7/1/19	ALL	EEG/telemetry surgery	Animal model + rhesus monkeys + telemetry + surgery	☐	☒	☒	☒
Scopus	7/1/19	ALL	Conflict Procedure	Animal model + rhesus monkey + conflict	☐	☒	☒	☒

Scopus	7/1/19	ALL	Conflict Procedure	Conflict + rhesus monkey + alternative	☒	☒	☒	☒
Scopus	7/1/19	ALL	I.V. Self-administration	Animal model + rhesus monkey + self-administration	☐	☒	☒	☒
Scopus	7/1/19	ALL	I.V. Self-administration	Alternative + rhesus monkey + self-administration	☒	☒	☒	☒
Scopus	7/1/19	ALL	I.V. Self-administration	Rhesus monkey + self-administration + drug	☒	☒	☒	☒
Scopus	7/1/19	ALL	Surgery (catheter)	Alternative + rhesus monkey + surgery + catheter	☐	☒	☒	☒
Scopus	7/1/19	ALL	Surgery (catheter)	Animal model + rhesus monkey + surgery + catheter	☐	☒	☒	☒
Scopus	7/1/19	ALL	Surgery (catheter)	Rhesus monkey + surgery + catheter + refinement	☐	☒	☒	☒
Scopus	7/1/19	ALL	I.M. injection	Animal model + rhesus monkey + intramuscular injection	☐	☒	☒	☒
Scopus	7/1/19	ALL	I.M. injection	Alternative + rhesus monkey + intramuscular injection	☐	☐	☒	☒
Scopus	7/1/19	ALL	Blood Draws	Alternative + rhesus monkey + blood draw	☐	☒	☒	☒
Scopus	7/1/19	ALL	Blood Draws	Animal model + rhesus monkey + blood draw	☐	☒	☒	☒
Scopus	7/1/19	ALL	Tail dip procedure	Animal model + rhesus monkey + tail dip	☐	☒	☒	☒
Scopus	7/1/19	ALL	Tail dip procedure	Alternative + Rhesus monkey + tail dip	☐	☐	☒	☒
Scopus	7/1/19	ALL	Tail dip procedure	Rhesus monkey + antinociception	☒	☒	☒	☒
Scopus	7/1/19	ALL	Vaginal swabs	Animal model + rhesus monkey + menstruation	☒	☒	☒	☒
Scopus	7/1/19	ALL	Vaginal swabs	rhesus monkey + vaginal swab	☐	☐	☒	☒
Scopus	7/1/19	ALL	Vaginal swabs	Alternative + rhesus monkey + vaginal swab	☒	☒	☒	☒
Scopus	7/1/19	ALL	Sleep Architecture	Sleep architecture + rhesus monkeys	☐	☒	☒	☒

Scopus	7/1/19	ALL	Sleep Architecture	Alternative + sleep architecture + rhesus monkeys	☒	☒	☒	☒
Scopus	7/1/19	ALL	Telemetry/Sleep	Telemetry + sleep + rhesus monkeys	☐	☒	☒	☒
Scopus	7/1/19	ALL	Telemetry/sleep	Alternative + telemetry + sleep + rhesus monkeys	☒	☒	☒	☒
Scopus	7/1/19	ALL	EEG/telemetry Surgery	Alternative + rhesus monkeys + telemetry + surgery	☒	☒	☒	☒
Scopus	7/1/19	ALL	EEG /telemetry surgery	Rhesus monkeys + telemetry + surgery	☐	☒	☒	☒
Scopus	7/1/19	ALL	EEG/telemetry surgery	Animal model + rhesus monkeys + telemetry + surgery	☐	☒	☒	☒

Narrative

Below, provide a brief summary of any articles that were identified in the search and how these studies relate to the current animal protocol. The narrative must discuss what efforts were made to REDUCE animal number and REFINE experimental procedures to reduce or eliminate pain and distress to the experimental animals, as well as whether there are alternatives that could REPLACE the use of animals. Interaction with peers and educational materials may be used to supplement discussion of literature searches.

Summary of articles:

The literature searches described above indicate that addiction researchers are using self-administration models of reinforcement to investigate the behavioral pharmacology/abuse-related effects of drugs. However, relatively few researchers have been evaluating the abuse-related effects of benzodiazepines in rhesus monkeys (e.g., ~2% of total "Animal model + rhesus monkey + self-administration" hits were associated with benzodiazepines, with all hits being publications from the [redacted] laboratory). The literature searches also emphasize that very few laboratories have been investigating sleep parameters in rhesus monkeys using implantable telemetry/polysomnography. The search for "Telemetry + sleep + rhesus monkeys" only engendered 7 results, and none of them were associated with benzodiazepines. These numbers indicate that benzodiazepine self-administration and telemetry/polysomnography sleep evaluation in monkeys, as proposed here, are not duplicative and represent an unmet need, given that these models are the "gold standard" of abuse liability and sleep assessment, respectively.

These searches also clearly emphasize that there are no alternatives to the proposed procedures. Self-administration studies, as well as other cognitive/operant/observable behavior studies, including the conflict and tail-dip procedures, require intact, behaving organisms. Search hits that included the terms "in vitro" or "alternative" were from studies that used these techniques to supplement behavior data, not to replace it; or were on an unrelated topic that happened to use these words/phrases.

Reductions in animal number:

Our primary strategy to reduce the number of monkeys is by using a within-subjects experimental approach or mixed-factor approach with within-subjects factor. For within-subjects designs each animal serves as its own control. This approach permits scientifically meaningful results to be obtained with fewer animals than would be required with other types of designs (e.g., an exclusively between-subjects approach). A second strategy that we use is to, whenever possible, re-use monkeys in our studies. Thus, once a particular experimental phase is completed; we will test the same monkey in subsequent experimental phases.

Refinements to methods to reduce distress:

Across all procedures, doses of test drugs/compounds have been chosen to be those with the least likelihood of producing dependence, withdrawal, and other adverse side effects in subjects. Additionally, we also have selected experimental parameters (e.g., limited session duration, schedule "time outs" between drug availability, having drug availability scheduled every other day) to reduce the likelihood of observing dependence and withdrawal with benzodiazepines. Importantly, we have included monitoring/behavioral scoring periods by technicians trained to recognize species-typical and drug-induced behaviors with the goal of identifying as early as possible side effects that may require intervention (e.g., withdrawal symptoms). Should mild withdrawal-like indications develop, diazepam (1 - 3 mg/kg or to effect; b.i.d.; i.m.) will be administered immediately to alleviate the physical symptoms, and benzodiazepine exposure will be ended by gradually reducing the dosage over a period of weeks to avoid possible precipitation of more severe symptoms. CCR veterinarians will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

Importantly, one of the methods proposed in this protocol is the use of subcutaneous vascular access ports attached to implanted intravenous catheters. This represents a refinement to our research program in that this new methodology allows us to move away from the jacket/tether/swivel and move towards more refined methods of intravenous drug delivery using a subcutaneous system for monkeys who do not need to be administered drugs in the home-cage. By eliminating the tether apparatus, there is also a possibility of working towards socialization/pair-housing in this subgroup of animals in the future, which ultimately would improve the animals' well-being.

The use of EEG/EOG/EMG telemetry technology is also a significant refinement of the activity monitor (actigraphy) approach. In addition, this technology represents a refinement compared to other similar technologies to investigate EEG/EOG/EMG patterns in rhesus monkeys. Telemetry implants permit the recording of EEG, EMG, and EOG signals under physiological conditions by limiting potential stress and allowing animals to freely move in their home-cages. We use a totally implantable telemetry system, which has no external connectors or cables; thus, in addition to no restriction of movement, there are no exit wounds, reducing the risk of infection. Importantly, the Physiotel Digital telemetry system from Data Sciences International (DSI) is compatible with social housing, also allowing for the possibility of working towards socialization/pair-housing in this subgroup of animals in the future. Finally, in addition to sleep measures, with this model we also hope to be able to capture the same patterns observed in humans regarding the effects of anxiolytic doses of benzodiazepines on EEG-derived brain wave activity. If that is the case, we will be able to screen drugs for anxiolytic effects by using EEG, which would ultimately minimize the use of the conflict procedure in our laboratory,

representing another refinement to our research program.

Animal **Replacement**:

Because we are studying whole-organism behavior/cognition/species-typical behavior, we cannot conduct our studies with tissue or cell lines, and there are no currently available replacements to the use of animals in experimental settings by use of computer simulation. In fact, computer simulation generally relies-and is only as good as-data generated by the types of studies in this protocol. Regardless, we will consider any alternatives should they become available.

Training and Qualifications

- PI

  Name► [redacted]
  Animal research experience ► 25+ years of experience with NHPs

  Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	[redacted] has 25+ years of experience conducting behavioral and pharmacology research with non-human primates, and developed the current research program contained in this protocol. He has experience with all procedures involved in running this lab.
Behavioral testing	
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	

- Other research personnel (copy the lines below for each individual listed as personnel on protocol)

  Name► [redacted].
  Animal research experience ► 25+ years of experience with NHPs

  Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	[redacted] has over 25 years of experience conducting behavioral and pharmacology research with non-human primates, and has experience with all procedures involved in this protocol. [redacted] is a trained primatologist, receiving her Ph.D. from [redacted] at U Massachusetts – [redacted] is a leading primatologist, having been trained by [redacted].
Behavioral testing	
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	

Name► [redacted]
Animal research experience ►10+ years research experience, 5 years with NHPs.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] has an MS in behavior analysis and 10+ years of experience conducting behavioral pharmacology research, with the last 5 in NHPs. She has experience with all procedures involved in the [redacted] lab NHP program and manages both the [redacted] and [redacted] labs.

Name► [redacted]
Animal research experience ► 15+ years of research experience, over 10 with NHPs.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] has 10+ years of experience working with NHPs, and currently conducts research in his own NHP laboratory at UMMC. He has experience with all relevant procedures, and may step in to assist the [redacted] lab from time to time as needed given the closeness of all NHP laboratories in both physical space and areas of research focus.

Name► [redacted]
Animal research experience ► 13+ years of research experience, 7+ years of NHP experience.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding	[redacted] has over 7 years of experience working with NHPs at UMMC. She was trained by [redacted], [redacted] and [redacted], and currently has her own NHP research lab. She has experience with all relevant [redacted] procedures and may step in to assist from time to time as needed given the closeness of all NHP laboratories in both physical space and areas of research focus.

NHP Chairing	

Name► [redacted]
Animal research experience ► 20+ years of rodent research experience, 13+ with NHPs.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] has over 13 years of experience working with NHPs, including time spent working with [redacted] and [redacted] at NEPRC. Prior to moving to the US, [redacted] worked at the Swiss Federal Institute. She has been thoroughly trained on all relevant procedures performed in the [redacted] lab.

Name► [redacted]
Animal research experience ► 7+ years of rodent research experience, 5+ years of research experience with NHPs

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] is a post-doctoral fellow working in the [redacted] lab. In addition to previous rodent research experience, she received a Ph.D. in neuroscience from Emory University, where she worked with NHPs prior to coming to UMMC. She therefore had a developed NHP research skill set prior to UMMC. She has been trained and is proficient on all NHP-related procedures performed in the [redacted] lab.

Name► [redacted]
Animal research experience ► 8+ years of research experience, 3+ years with NHPs.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	Trained to assist in surgery.
Behavioral testing Observations	[redacted] is a post-doctoral fellow working in the [redacted] lab. In addition to previous behavioral research with rodents and

Catheter flushing/maintenance	pigeons, she is also a board-certified behavior analyst. She has been thoroughly trained on any NHP procedures relevant to her work, and is primarily responsible for behavioral observations.
Enrichment & Feeding	
NHP Chairing	To be trained

Name► [redacted]
Animal research experience ► 2+ years of research experience with NHPs & rats

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	To be trained
Behavioral testing	[redacted] is a research tech that has been working with the [redacted] and [redacted] labs for 1 year (and spent the previous 1+ years with us as a volunteer and SURE student), so he has been trained on all basic relevant tasks in the lab. He will be trained on surgeries and chairing in the near future.
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	To be trained

Name► [redacted]
Animal research experience ► 2+ years of research experience (rats & NHPs, [redacted] lab)

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	[redacted] is a graduate student working in the [redacted] and [redacted] labs. He previously worked as a volunteer and SURE student in the [redacted] lab (primarily with rats), and will require training on all NHP-related tasks. He has been trained to do basic enrichment and
Behavioral testing	

Observations	feeding, is currently training on behavioral observations and behavioral testing, and will eventually be trained on surgery and chairing. See “training to be provided” below for more details on how training will take place.
Catheter flushing/maintenance	
Enrichment & Feeding	Trained by [redacted]
NHP Chairing	To be trained

Name► [redacted]
Animal research experience ► 4 years of experience working with CCR, 3 years of experience working in the [redacted] laboratory.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	To be trained by [redacted] staff – will only be involved in [redacted] surgeries under special circumstances.
Behavioral testing	[redacted] is the lab manager for the [redacted] and [redacted] labs, and given the closeness of all NHP labs he may at times assist on procedures or tasks related to [redacted] NHPs. He has experience working for CCR (husbandry) prior to working for the [redacted] and [redacted] labs.
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	

Name► [redacted]
Animal research experience ► Current graduate student in the [redacted] lab – research experience with both rats and NHPs.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	[redacted] is a graduate student in the [redacted] lab. The primary focus of his current work is with rats, but he does do some NHP studies. Given the closeness of the [redacted] [redacted] and [redacted] labs, he may at times assist with NHP-related tasks such as feeding or monitoring. He will only be asked to help with tasks he has been thoroughly trained on by his own lab (typically feeding/enrichment, occasionally assisting on surgery, etc.).
Behavioral testing	
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	

Name► [redacted]
Animal research experience ► Approx. 1 year NHP experience ([redacted] lab tech)

Qualifications to perform specific procedures

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

Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] is a research tech for the [redacted] lab. Given the closeness of the primate labs, she may at times assist with NHP procedures and tasks in [redacted] rooms (e.g., train on observations, assist on surgeries, enrichment/feeding). Training will be provided by the [redacted] lab, and she will only be asked to help out in rare instances and on tasks that she has been deemed fully proficient by her own lab on.

Name► [redacted]
Animal research experience ► MSRP student, no previous experience

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations Catheter flushing/maintenance Enrichment & Feeding NHP Chairing	[redacted] is an MSRP student with no previous NHP research experience. He will primarily be focused on rodent work within the [redacted] lab, but may assist in some general NHP tasks in order to gain experience. He will need to be trained on all tasks and procedures. Given the closeness of the [redacted] and [redacted] labs, he may train in both labs.

Name► [redacted]
Animal research experience ►Head of UMMC Animal Behavior Core

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Sleep Scoring EEG Telemetry	The [redacted] lab (specifically [redacted]) has recently developed a set of sleep scoring and EEG telemetry research studies. Given [redacted] many years of experience with sleep research, he may be involved in portions of this work, be present for EEG surgeries, etc., to provide valuable input.

Name► [redacted]
Animal research experience ► No animal research experience ([redacted] tech)

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring Behavioral testing Observations	[redacted] is a research tech in [redacted] lab. We are collaborating on the creation of a library of blood samples/genetic data from our NHP colony, and he is interested in being present during blood draws. His help in the lab will not extend past his assistance in this process.

Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	

Name► [redacted]
Animal research experience ► 1+ summers in SURE program w/[redacted] lab

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	SURE student/undergrad – will not train on Sx.
Behavioral testing	[redacted] is a SURE student who worked with the [redacted] lab summer 2018 and has returned for Summer 2019. He has been trained on all basic NHP tasks (enrichment, feeding, etc.), and is currently training in observations.
Observations	
Catheter flushing/maintenance	
Enrichment & Feeding	
NHP Chairing	To be trained

Name► [redacted]
Animal research experience ► 1+ summers in SURE program w[redacted]

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	SURE student/undergrad – will not train on Sx.
Behavioral testing	[redacted] is a SURE student who worked with the [redacted] lab summer 2018 and has returned for summer 2019. He has been trained on basic NHP tasks (enrichment & feeding, behavioral testing, etc.). He is primarily a student in the [redacted] lab but may assist with [redacted] work.
Observations	
Enrichment & Feeding	
Catheter flushing & Maintenance	To be trained (Primarily a [redacted] student, so may not train on this)
NHP Chairing	To be trained (Primarily a [redacted] student, so may not train on this)

Name► [redacted]
Animal research experience ► None (SURE Student – Summer 2019)

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Catheter surgeries & Post-surgical monitoring	SURE Student – will not train on Sx.
Behavioral testing	

Observations	[redacted] is a SURE student working in the [redacted] lab. She has been trained on enrichment & feeding, and is currently training on behavioral testing, observations, and catheter maintenance by [redacted] and [redacted].
Enrichment & Feeding	
Catheter flushing & Maintenance	
NHP Chairing	To be trained

- **Training to be provided.** List here each procedure 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, enter "N/A"

Surgical Training: Currently, [redacted] and [redacted] are trained to perform IV Catheter surgeries (in addition to: [redacted]). [redacted] is currently training to perform IV catheter surgeries. Training typically involves the trainee assisting an experienced surgeon and gradually completing more and more steps of the surgery under the direct supervision of one of the trained surgeons. This supervision and training will continue until trainees can complete an entire surgery start to finish without assistance from the trained surgeons.
NHP Chair Training: [redacted] is currently the most experienced at NHP chairing in the [redacted] lab, and therefore any training on this procedure will take place with her. Training a new individual to chair monkeys involves training in positive reinforcement and shaping of behavior, as well as safety precautions given that it involves taking a monkey out of its home cage. Trainees will begin by observing [redacted], and fade in the amount of physical assistance they provide while [redacted] fades out her own part in the chairing until the trainee is able to complete the process on their own.
Behavioral Testing and Observations: Behavioral testing and observation studies involve learning a variety of skills including behavioral coding, using MedPC and other computer programs, and a knowledge of how to hook up certain sets of equipment, such as self-administration panels. Any persons requiring training will work closely with an individual who is already proficient on the task. For observation training, individuals must meet 90% reliability criteria (i.e., observation data must match 90% of the trained observer's data) in order to be considered trained.

Certification of the Principal Investigator:

Signature certifies that the Principal Investigator will conduct the project in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations, and UMC policies governing the use of live vertebrate animals for research and teaching purposes. The procedures involving animals will be conducted by trained or experienced personnel or under the direct supervision of trained or experienced persons. It is understood that IACUC approval is valid for a period of 12 months following the date of original approval and must be renewed annually for continued approval. I understand there is a 3-year requirement for full protocol rewrite. It is further understood that should this project be submitted for external funding, the information presented on the UMMC Animal Activity Protocol form accurately reflects the animal use in the full grant application.

X [redacted]

Signature of Principal Investigator (Paste digital copy of signature)

Approval by the Attending Veterinarian:

[redacted]

Signature of Attending Veterinarian or Designee

Approval by the Institutional Animal Care and Use Committee:

X [redacted]

Signature of IACUC Chair or Designee

Appendix A Non-Human Primate Environmental Enhancement/ Enrichment

This appendix must be appended to each protocol involving the use of nonhuman primates.

Nonhuman primates must have their physical environments enhanced/enriched by providing means of expressing non-injurious, species-typical activities. The *Animal Welfare Act* (9 CFR 3.81) states that research facilities "must develop, document, and follow an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates".

The default position of USDA and OLAW is that non-human primates must be socially housed. The Guide (2011) states, "*… nonhuman primates should normally have social housing (i.e., in compatible pairs or in larger groups of compatible animals)".* Exemptions to the social housing requirement must be based on **strong scientific justification** approved by the IACUC or for a specific veterinary or behavioral reason.

The Center for Comparative Research provides an active plan of environmental enrichment that includes cage complexities (tunnels, barrels), social interaction, fruit/vegetable supplements, foraging, and manipulative devices/toys. Unless otherwise, specified, the CCR will provide all available forms of enrichment.

1. Enrichment Techniques

Are there any enrichment forms/techniques that are included in this protocol?

☐No ☒Yes

2. Description

Describe the above techniques.

In general, CCR and the lab collaborate to provide an active plan of environmental enrichment. Acceptable forms of enrichment include: social interaction (visual, auditory, grooming interactions), fruit/vegetable supplements to daily feeding, foraging, mirrors, and manipulative devices/toys. CCR provides mirrors, toys, and fruits/vegetables, and the lab provides forage that can be added to bedding daily as a form of enrichment. A television and movies are also provided to each room on a weekly basis when available. Enrichment is documented in writing by the person that gives it to the NHPs on both the room door sheet and daily log sheets.

3. Exemption from Enrichment

Are there any forms of enrichment/enhancement that should not be used in this study?

☐No ☒Yes

4. Justification for Exemption

If Yes, provide complete justification for this exemption.

Exemption from social/pair housing:

These studies are conducted in the monkey's individual living quarters. For these studies, the cages have been modified so that experimental equipment can be mounted to the side of the cage unit. Monkeys self-administer intravenous (i.v.) injections of drugs/compounds. The i.v. catheter exits the monkey's back and is threaded through a tether attached to a swivel on the side of the cage, and monkeys wear custom jackets to protect the catheters. Another monkey in the cage would very likely interfere with the jacket, tether, and/or swivel in such a way as to put the catheter at risk of damage or even removal. Moreover, the presence of another monkey in the cage would interfere with the performance of the monkey on experimental tasks, or alter dramatically the behavior of the monkey during observation sessions, thereby seriously compromising the validity of our research. In most cases, however, individual cages will be grouped together in colony rooms in order to allow visual, auditory, and olfactory contact with other monkeys. Tactile contact between cages may also be available depending on the compatibility of individual monkeys.

Exemption from other forms of enrichment:

Monkeys in this protocol are given foraging treats and room enhancements (e.g., radio, movies, interaction with humans, etc.). However, given the behavioral nature of our research and the specialized home cages used for some studies, we request an exemption from enrichment during experimental sessions that would interfere with the conduct of research. There is ample scientific evidence that the availability of alternative behaviors during an experimental session can alter drug taking (e.g., Nader & Woolverton, Psychopharmacology 105:169, 1991). Therefore, enrichment needs to be removed from home cages during some behavioral sessions so that manipulation of enrichment devices cannot interfere with the behavior being studied, i.e., the manipulation of levers. During this period, the environment is enriched by the opportunity to manipulate levers and receive reinforcement.
Additionally, a small number of NHPs tend to engage in self-injurious behavior if a mirror is present in their cage. For these monkeys, a mirror is not a part of the daily environmental enrichment plan.

Appendix A Non-Human Primate Environmental Enhancement/ Enrichment

Appendix C Surgery & Management of Surgical Pain and Distress

1. **Complete description of surgical procedures** – List details for each surgical approach noted in question #16.

Surgical site preparation
FOR CATHETER SURGERIES: Surgery is performed in a fully-equipped operating suite (CCR surgical suites, [redacted]) using appropriate anesthetic agents and analgesics. Monkeys are initially given atropine (to reduce secretions) and ketamine in their home cage prior to surgery, then moved to the surgical prep room. Aseptic preparation of the surgical site (limb or neck) and midscapular exit site occurs in the prep room. Hair is removed using clippers followed by a disposable razor, if needed. The surgical site is prepared with three alternating applications of surgical scrub (betadine or chlorhexidine) and 70% alcohol or similar. After arrival in the operating room, a final scrub is performed and sterile drapes are placed. Inhaled isoflurane in oxygen is administered for the duration of the surgery. **FOR EEG SURGERIES:** Surgery is performed in a fully-equipped operating suite (CCR surgical suites, [redacted]) using appropriate anesthetic agents and analgesics. Monkeys are initially given atropine (to reduce secretions) and ketamine in their home cage prior to surgery, then moved to the surgical prep room. Aseptic preparation of the surgical site (dorsum, head, neck and canthi of either the right or left eye) occurs in the prep room. Hair is removed using clippers followed by a disposable razor, if needed. The surgical site is prepared with three alternating applications of surgical scrub (betadine or chlorhexidine) and 70% alcohol or similar. After arrival in the operating room, the animal is positioned in sternal recumbency with access to the dorsum and head using patient positioning devices (i.e. Versaform pad or stereotaxic frame). Additional towels/pads may be used to support the animal as needed. Arms will be positioned so the neck area isn't tense or bunched, either back or out to the sides. Corner drapes are placed over each ear and the face and corner drapes are stapled down to create defined working area on head (corner drapes also help prevent excessive amounts of saline from running onto the animal). The paper drape will be stapled to the skin using surgical skin staplers. Staples will be removed using a staple remover or mosquito hemostats at the time of drape removal. The animal will be positioned in such a way that all leads (EEG/EMG/EOG) can be placed without having to reposition it – ventral recumbency, with the head elevated. Sterile drapes are placed on the entire animals. Inhaled isoflurane in oxygen is administered for the duration of the surgery.
Surgical approach
FOR CATHETER SURGERIES: Chronic intravenous catheters are implanted following the general procedure described by Carey & Spealman, Current Protocols in Pharmacology, pp. 10.5.1 – 10.5.15 (1998), updated by [redacted] et al., Current Protocols in Pharmacology, Unit 9.2.1 (2005). For the catheter implantation, a small skin incision is made over the femoral, jugular, or brachial vein of the anesthetized monkey. The vein is exposed by blunt dissection, tied off to prevent blood loss, and an incision is made in the vein to accept one end of a polyvinyl (or other medical grade material) catheter. The catheter is passed through the vein to the level of the right atrium and secured to the vein and adjacent muscle by sutures. This placement of the cathether to the right atrium is achieved by measuring and cutting the catheter based on the type of vein and the size of the monkey (measurement made from incision site to center of sternum). Once in place, the catheter is observed for several minutes to ensure that blood does not advance into the lumen, which would indicate that the catheter is too close to the heart. If this occurs, the catheter is removed, shortened, and re-implanted. Once the catheter is determined to be the appropriate length and is placed, it will be secured to the muscle at the incision site via two cuffs placed on the catheter line. Pulling back rather than removing and cutting would result in the catheter cuffs being out of place. Once the catheter is secured, the distal end of the catheter is passed subcutaneously through a hollow probe to exit the body in the midscapular region. From this point, the NHP may be fitted with a jacket and the

catheter threaded through a tether attached to said jacket, or a port will be implanted (see below).

PORT IMPLANTATION FOR CATHETER SURGERIES: After the hollow probe has been removed, the catheter is gently pulled to ensure the excess in the limb or neck is eliminated without disturbing the prep or contaminating the catheter. A sterile drape is placed over the catheter and exit site, and a non-sterile person turns the monkey back over so that the incision site can be accessed for suturing. The surgeon then will change into clean, sterile gloves in order to suture the incision and complete the subcutaneous vascular access port placement. After suturing of the incision on limb or neck is complete, a non-sterile person will turn the monkey back to a more sternal position to allow easy access to the back, and the back is re-prepped with betadine or chlorhexidine. New sterile drapes are placed to establish a sterile field around the exit site. A small skin incision is made starting at the point where the catheter exits the skin either lateral or medial to the exit point, depending on where the exit point is in relation to the spine, so that the port resides next to the spine on the side where the catheter was implanted. The incision is made perpendicular to the spine, with a catheter loop (4-6 cm long) placed cranial and the port caudal to the incision. The catheter is attached to the port, and the port is flushed with 2-3 ml of heparin lock flush solution using a Huber needle, and the port is placed in the subcutaneous pocket. After the port has been anchored to the underlying tissue at two positions on the skirt to keep it from flipping following surgery, the incision in the back is closed. An even more thorough explanation of details and step-by-step procedures is listed in our Port surgery SOP, which is attached to this protocol.

FOR EEG SURGERIES:

For the device implantation (implant device measures: weight 56 grams, volume 29cc, dimensions 59 x 38 x 15 mm), a skin incision is made lateral to the spine beginning just caudal to the scapulae extending caudally. The skin is retracted, as needed, and the surgeon bluntly dissects between muscle fibers to get through the latissimus dorsi muscle. Blunt dissection is typically used between muscle fibers to create the pocket between the two layers. A small amount of sharp dissection with a scalpel may be alternatively used. The incision and pocket are stretched out with moistened fingers. The device is placed under the latissimus dorsi muscle, and the pocket size is increased as needed to accommodate device. The surgeon then visualizes approximately how far caudally the antenna will extend and a small stab incision is made slightly distal to this point. A trocar and cannula are passed between the main implant pocket and the stab incision and the cannula is used to guide the antenna in a straight path distally. Non-absorbable suture (3-0 Ethibond) is used to tack the device to the underlying fascia. Incision should begin caudal to the browbone and extend to approximately inion. The incision may need to be extended to expose some neck muscle where the leads will be tacked. The leads are marked to distinguish channels if necessary. A trocar and cannula are used to tunnel the biopotential leads from the dorsal pocket to the cranial incision. The cranial incision covers the majority of the skull (from near nasion to inion) in order to get adequate exposure. Leads (EEG/EMG/EOG) are routed altogether, separating only when they go to either the cranium, neck or the orbit, without the need for repositioning the animal. A small loop of lead is left at this incision and incision is packed with moistened gauze. All gauze will be removed before closing. Some excess lead length will be left to provide strain relief and prevent the leads from being pulled out of place with normal postural changes and activity.

Two EEG leads (2 channels, 2 leads each) will be placed in the skull, one in a central derivation (C4) and the other in an occipital dervation (O2) (please see attachments “DSI EEG Positioning” and “10_20 EEG Positioning” for a detailed description of the location of scalp electrodes based on the human 10/20 positioning system. scalp electrodes based on the human 10/20 positioning system). For the placement of the EEG leads, all muscle, connective

tissue, and periosteum covering the skull is reflected with periosteal elevators. Gelpi retractors are inserted at the rostral and caudal aspects of the incision. Measurements, as needed, are made on skull with sterile tape measure and insertion sites are marked with sterile marker. The lead is then prepared by trimming the leads to an appropriate length and trimming the insulation off the distal 3-5 cm lead. A piece of 4-0 Prolene is tied around the distalmost portion of the insulation to help prevent it from slipping back. The exposed wire is bent in to a tiny loop and secured with a mosquito hemostat, and a screw is placed inside the loop. If the head of the screw fits through the loop, the loop is made tighter. Another piece of 4-0 Prolene is tied around the loop to secure it. A drill is then placed firmly against the skull at a perpendicular angle, and the skull is perforated in the desired locations. The holes should pass completely through the bone, but not perforate the dura. Lavage with saline is performed the entire time drill is in use. Screws are placed in holes perpendicularly to the skull. If the screw doesn't go down all the way or seems to stop advancing, the hole may be incomplete. Drill again through the same hole as needed. As the screws are tightened down, create a loop of lead on the surface of the skull around the screw to provide strain relief. Excess wire from loop is trimmed. Skull is etched with scalpel blade around the screws before placing the dental acrylic. All exposed wire and screw is covered with dental acrylic, dental acrylic is smoothed and lead material is pushed down against the surface of the skull. A non-sterile assistant aims the UV cure light at the acrylic and activates at least two times to harden the acrylic. Personnel should not look at the UV light when it is on. Optional: a tack suture is placed through the muscle on the neck with 4-0 nonabsorbable suture (Prolene) and the tails are tied around two of the leads to help prevent slippage, ensuring that enough slack remains to allow for normal head range of motion. The process is repeated for the other set of leads.
For the placement of the EMG lead (1 channel, 2 leads), the cranial skin incision is extended as needed, ~4 cm, to expose the longitudinal muscle of the neck (e.g. trapezius). An area of the muscle that can accommodate lead attachment and allow the leads to lie flat under the skin is selected. The leads are cut to an appropriate length, leaving some excess to allow for growth and/or postural changes. The wire lead is exposed by making a circumferential cut to remove the silicone covering from the last 5-6 mm of the lead. The stripped insulation is set aside for later. A piece of non-absorbable suture is tied around the distal silicone a few millimeters from the end to prevent fluid migration (body fluid can migrate up the lead underneath the silicone insulation material, and it could potentially enter the implant via this route; therefore, a piece of suture is placed circumferentially around the insulation to prevent this from happening while the implant is in the animal). Using an 18- gauge needle, approximately 4 mm of muscle tissue is tunneled through perpendicular to the long axis of the fiber bundles. The needle should pass from the outer margins of the animal toward the midline. The exposed wire is passed into the lumen of the needle so that, as the needle is withdrawn, most of the wire is left embedded in the muscle. Approximately 1 cm of the distal end of the wire should be exteriorized from the muscle. The saved piece of insulation is placed onto the terminal end of the lead wire, secured with a suture tie and trimmed to length so that the insulation extends slightly beyond the wire. This will anchor the lead in place and protect the animal's skin from the cut end of the wire. The process is repeated for the other lead. Both leads should be placed approximately 4 mm apart within the same group of muscle fibers, ensuring that the exposed portion of the leads from each channel do not make contact as that could cause shorting of the signal.
For the placement of the EOG lead (1 channel, 2 leads), the wire lead is exposed by making a circumferential cut to remove the silicone covering from the last 5-6 mm of the lead. There is no real incision for the placement of the EOG leads. The leads are routed subcutaneously to a position adjacent to the lateral and medial canthus of the right eye, respectively. The periosteum of the orbit at those locations is then elevated and the electrodes affixed to and under it with non-absorbable suture material in a simple interrupted pattern. The exposed wire

is completely covered with periosteum and possibly a small portion of conjunctiva. The electrodes may be additionally fixed with a tissue adhesive.

Wound closure method, materials, and removal plan

Catheter surgeries: The incision is sutured using a combination of sub-cuticular and external suturing techniques (e.g., sub-cuticular continuous suture followed by horizontal mattress sutures). An appropriate absorbable material is used for all suturing (e.g., Maxon/Biosyn, vicryl). External sutures may be done with non-absorbable material which will be removed one week post-surgery. Catheters are flushed regularly with saline/heparin solution (100-150 u/mL) and sealed with stainless steel obturators or knots when not in use. Either a port is surgically implanted in the monkey's back or the monkey wears a custom-fitted nylon mesh jacket at all times to protect the catheter. Catheters are expected to remain patent for 6 to 36 months, although they may fail sooner or last far longer than that.

Catheters that become occluded or dislodged will be replaced by re-implanting a new catheter in the original vein. If the catheter cannot be replaced, the monkey is sutured as described above and another vein will be catheterized after a minimum of 1 week recovery period between surgeries. Up to 8 vessels (2 internal jugular veins, 2 external jugular veins, 2 brachial veins, 2 femoral veins) are used for these surgeries, depending on the disposition of the monkey. Sometimes the catheter is broken at the exit site and will, in the majority of cases, reenter the body. If this occurs, the monkey is prepared as described above for aseptic surgery, but placed on the abdomen. After surgical preparation of the area around the last exit site, a small incision is made, and the catheter is re-exposed using blunt dissection. A sterile metal connecting pin is used to attach additional catheter material as needed. For this procedure, the monkey's pre- and post-operative care is identical to catheter implantation.

Vascular Access Ports (Catheter surgeries): Both incisions (limb/neck and back are sutured using the same suturing techniques described above for catheter surgeries. The subcutaneous vascular access port should be removed/replaced any time there are signs of irritation or inflammation around the port area or along the catheter track. Signs which may warrant port movement include: thinning of skin over the port, increased redness, swelling, warmth on or around the port or catheter track; signs that the animal is scratching or picking at the port or catheter track. As long as there are no signs of catheter infection, ports can be replaced without necessarily having to replace the catheter. A 1-week recovery period is provided between port removal and new port placement, with the catheter being placed subcutaneous until the placement of a new port. For these procedures, the monkey is prepared and monitored pre- and post-operatively as stated above for catheter surgeries.

EEG Surgeries: The animal's back (device implantation) is sutured in two layers. The subcutaneous layer is sutured with 4-0 absorbable suture on taper (BioSyn, Vicryl) in a simple continuous pattern. Skin is then closed with intradermal pattern using 4-0 absorbable suture on cutting (Vicryl). Stab incisions are closed by suturing the skin layer, as described above. The muscle layer on the head incision is sutured using and a combination of interrupted tension-relieving suture patterns (3-0 BioSyn on taper) and/or a simple continuous pattern (using 4-0 BioSyn, taper). Subcutaneous tissue is then closed in a simple continuous pattern with 4-0 BioSyn on a tapered needle. Skin is closed in an intradermal pattern with 4-0 BioSyn on cutting.

Implants will remain in the animals and in use until the batteries die out. The battery life of the implants used in our studies (L04 devices) is 95 days or higher when recording uninterruptedly. In most cases, the devices will be turned on and off remotely through radio frequencies, and based on how frequently we plan on having the systems on, we expect batteries to last ~18-24 months under our experimental conditions (we currently have 4

animals whose implants were placed over 12 months ago and the batteries of all 4 implants are still operable).
Once batteries are no longer operable, the implant body (device) can be replaced with a new one and previously placed leads can be kept and attached to the new implant if signals are still good. In case of implant adhesion and inability to remove the old implant, a new implant will be placed on the opposite side of the animal's torso. Briefly, a skin incision is made lateral to the spine, at the original implant site, and the original implant is identified through blunt dissection. The leads are cut and detached from the original implant. A new incision is made on the opposite side of the animals' torso, lateral to the spine beginning just caudal to the scapulae extending caudally. The leads are routed subcutaneously from the first incision to the second incision site. Once externalized at the second incision site, the leads are attached to the new implant, and the device is positioned under the latissimus dorsi muscle as described above for the original implant placement. We anticipate that most monkeys will have a second implant.
If signals from leads are no longer good, we will not attempt to replace them. Instead, in consultation with CCR veterinarians and DSI consultants, new leads will be implanted as needed, using the surgical procedures described above. If any of the second leads fail, then we will halt these studies and will re-assign the monkey for experiments not requiring telemetry, in consultation with CCR veterinarians.
Please see attached table containing possible complications of EEG surgeries and how we intend to handle them.

2. Provide a complete formulary of medications related to surgical procedures:

	Agent	Dose	Route	Frequency /Duration	Pharmaceutical Grade
Pre-anesthetic	Atropine	0.02-0.05 mg/kg	i.m.	Once	☒Yes ☐No
Pre-anesthetic	Ketamine	10-20 mg/kg	i.m.	Once, then as needed	☒Yes ☐No
Pre-operative steroids	Dexamethasone	1-2 mg/kg	i.m.	Once, at time of preparation for surgery	☒Yes ☐No
Analgesic	Buprenorphine SR (or buprenorphine)	BUP SR: 0.045 mg/kg BUP: 0.005-0.03 mg/kg	BUP SR: s.c. BUP: s.c. or i.m.	BUP SR: Once BUP: Every 8-12hr for 72 hours	☒Yes ☐No
Post-operative analgesics	Carprofen	2.0-4.0 mg/kg	s.c. or p.o.	Every 24hr for 3 days post-op	☒Yes ☐No
Anesthetics	Isoflurane	1-2% (in O_2)	Inhaled	Throughout procedure	☐Yes ☐No
Fluid/blood replacement	Saline	0.9%	i.v.	As needed	☒Yes ☐No
Antibiotics	Cephalexin	20 mg/kg	i.v., i.m., p.o.	B.I.D./ 5-7 days	☒Yes ☐No

For non-pharmaceutical-grade compounds:

a. Justify the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

N/A

b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

N/A

3. Anesthesia

a. Who will conduct the anesthesia procedure(s)?

CCR veterinary staff (veterinary technician or veterinarian)

b. Describe experience and training with anesthesia.

CCR veterinary technicians are certified veterinary technicians and/or have been extensively trained by CCR members who have significant experience with this procedure.

c. What criteria will be used to assess anesthetic depth and how will this be monitored?

Monitored by CCR vet staff (Criteria used: heart rate, respiratory rate, muscle tone, oxygen saturation, body temperature).

4. Aseptic Technique

a. What procedures will the surgeon use to prepare himself/herself for aseptic surgery?

Surgeons will prepare hands and arms with aseptic procedure (scrub in, use aseptic hand spray in room after scrubbing). Sterile surgical gowns will be worn, as well as sterile gloves. Face masks, eye protection, hair bonnets and shoe covers also will be worn. Assurance of proper technique will be assessed by the CCR veterinary staff prior to the initial surgery.

b. How will the instruments be prepared for aseptic surgery? (Sterile instruments must be used for each animal.)

All instruments and surgical packs will be autoclaved as appropriate. Equipment for surgery that is not appropriate for autoclaving (e.g., polyvinyl chloride catheter material) will be sterilized via the Ethylene Oxide (EO) sterilization unit housed within the UMMC dental school. Whenever possible, commercially-available sterile instruments and equipment will be used.

5. Location of Procedures

Where will the surgical procedures be conducted?

CCR surgical suites [redacted]

6. Post-procedural Care

a. Who will conduct and document post-procedural animal care (post-op analgesia, nursing care, etc.)? *Documentation will be checked at IACUC semi-annual inspection.*

Laboratory staff and CCR staff will conduct post-op monitoring in accordance with the CCR post-op monitoring form.

b. Include a plan of monitoring frequency, duration and intervals of post-op analgesia, nursing care, etc.

Monkeys are monitored continuously until extubation by CCR staff, after which lab staff monitor continuously until the monkey is sitting up on their own and responsive to their environment. Lab staff will administer post-operative drugs (antibiotics, analgesics) over the next 3-7 days in accordance with CCR recommendation(s). Analgesics and antibiotics are administered in close collaboration with the veterinary staff.

c. What is the expected time from end of procedure until animal(s) are returned to home environment?

No more than 1 hour.

7. Emergency Contacts

Provide emergency contact information (pager/phone number) for evenings or weekends concerning post-operative complications.

[redacted]

We list below potential post-operative complications with EEG/telemetry surgeries and how we will plan on addressing or preventing these. The majority of these potential complications are shared by virtually all chronic instrumentation surgeries. The risk of these complications can usually be reduced significantly by adequate planning preparation, careful technique, and attentive peri-operative care.

Potential Complications	**Mitigations**
Surgical Site Infection/Delayed healing	This is a risk of all surgeries. Steps that are taken to prevent include: • Strict aseptic technique • Prophylactic antibiotics • Appropriate tissue handling/surgical technique. This is one important reason why a large enough incision is critical. If the surgeons attempt to access the necessary areas of the skull without adequate access, unnecessary tissue trauma will be caused, which increases the risk of infection. Surgeons will also use surgical techniques such as buried suture patterns, so there are not external skin sutures/staples for the animals to disturb. • Healing at an incision takes place from side-to-side (rather than front to back). Therefore, a long incision heals at the same rate as a shorter incision, as long as appropriate tissue handling is performed at the time of surgery.
Implant complications (e.g. animal picking at implant, rejection of implant)	• The device is placed underneath a covering of skin and muscle. The intermuscular placement provides some “cushion” over the device and protects the skin, increasing animal comfort and substantially decreasing the chance of erosion of skin over the implant. • The fact that the incisions are all closed with buried suture, with no external sutures/staples, makes the incision less irritating to the animals, and makes it much more difficult for the animals to find anything to disturb.
Inadequate signal quality	• If there are placement issues at the time of surgery, or if the implant shifts out of place post-operatively, the signal quality may be compromised, and the animal may not be able to be included on the study. One of the concerns in the EEG surgery is ensuring that the exposed metal of the screw and wire are fully insulated under the acrylic material. This means that additional tissue has to be cleared from the area around where the leads are placed. This additional dissection is critical to ensure high quality signals and prevents wastage of animals with signal interference.

	• Telemetry signals are monitored throughout the surgery to assess and adjust the signals as necessary.
Post-operative pain	• The DSI surgical team will work with our CCR veterinary team to develop a pre-, intra- and post-operative analgesia and intra-operative anesthesia protocol tailored to these animals and surgeries to help maintain comfort. • The DSI surgical team will be available for telephone consultation, as needed, post-operatively when we're no longer on-site. • The DSI surgical team recommends multi-modal analgesia to block multiple different pain pathways and prevent the development of windup pain.
Neurologic complications	• A special neurologic drill with a stop on it will be used, so that the surgeons can stop at the precise depth (to the mm) to avoid damage to the brain • Anesthetic adjustments (e.g. altering respiratory rate to decrease carbon dioxide levels, elevating the head slightly, avoiding compression of the jugular veins) is recommended to increase the space between the skull and the brain to avoid trauma. • Anti-inflammatory medication will be administered to prevent swelling of the brain

DSI has extensive history of implanting rhesus monkeys with EEG (per surgical technique described in this protocol), ECG, blood pressure, core body temperature, and activity. Any complications seen in these animals are described below (note that this list was provided by DSI, and these complications did not develop in animals from the [redacted] lab).

Previous Complications	**Mitigations**
Implant Disturbance: One animal was maintained in quarantine after shipment to the researcher's site for approximately two months+. Toward the end of the quarantine period the animal was examined, and was determined to have picked through the skin and exposed the tip of the antenna.	• Surgical modification to tunnel the antenna to a small stab incision, rather than blindly tunneling under the skin. This allows surgeons to keep the antenna underneath a muscle layer for the majority of the length, thus protecting the underside of the skin from irritation, and prevents the antenna from bowing up under the skin. • Animals will be carefully monitored during the post-operative, recovery, quarantine and study period. Interventions such as novel enrichment may be added if self-injurious behavior is detected.
Stereotypical Behavior: One animal demonstrated circling behavior post-operatively	• Analgesic and anti-inflammatory protocol was assessed and updated • Animal was observed with personnel outside the room and was not demonstrating the behavior, so it was assumed that this animal was undergoing stereotypic behavior and not in pain or experiencing neurologic complications.

Appendix D Collection of Biological Samples from the Live Animal

Biological samples include blood collection, urine collection, ascites, tail tips for DNA, cerebrospinal fluid, biopsy, etc. Appendix D is completed for all sample collections from live animals, including under terminal anesthesia. Appendix D is not required for samples taken after euthanasia.

1. Indicate the body fluid or material to be collected.

Blood and vaginal discharge

2. Indicate the method and site of collection.

Blood: Will be collected from the femoral or saphenous vein using vacutainers and a new puncture for each sample. In some cases, the monkeys will be lightly sedated with ketamine (10-20mg/kg, see appendix F) for the collection of samples. In order to reduce the number of necessary sedations, blood may be collected when the monkey is already sedated for a planned health check or cage change.
For other samples, awake sampling will be required since (a) ketamine may interfere with the experimental endpoints and (b) behavioral tasks will occur soon after sampling (i.e., recovery time would interfere with the monkey's ability to perform behavioral tasks). For awake sampling, the monkey will first be trained to present the bottom part of a leg (i.e., below the knee) through the feeder hole on the front of the cage unit (which can be accessed without unlocking any cage doors), or by using the front door panel c combined with the squeeze-restraint mechanism in the cage, as well as gentle handling with food reinforcement. The method of "successive approximations" will be used, in which components of the task will be successively reinforced (e.g., sitting in front of the cage door results in an apple slice, then allowing the trainer to touch the leg will be required in order to obtain the apple slice, and so on). Using this technique, we have routinely performed awake blood draws on consecutive days for up to one month.

Vaginal Discharge: Will be collected for evaluation of cells consistent with different phases of the menstrual cycle. Awake sampling will be required for the same reasons described above for awake blood sampling. The monkey will be trained to present her hindquarters, and again the method of shaping or "successive approximations" will be used to train the animal to present her hindquarters, then allow the trainer to touch the skin near the vaginal opening with a cotton-tipped swab, and so on.

3. Indicate the volume of fluid or amount of material to be collected.

Blood: Up to 3ml per draw
Vaginal Discharge: A single cotton-tipped applicator

4. Indicate the frequency of collection.

Blood sampling for hormone levels: Blood samples are required for analyzing progesterone and estradiol determination, which is used to determine different

phases of the menstrual cycle in female rhesus monkeys. These may be collected for the duration of time the subject is on the study (i.e., our collection may be continuous and may occur with all subjects in the approved protocol). Samples may be collected daily for at least one complete cycle (~28 days). After this initial precise determination, sampling during a cycle may occur every 2 to 4 days. Further determination of the entire cycle may be necessary periodically throughout the study (approximately every 6 months) and at the end of the study to assess potential drug effects and/or changes in cycling. We will ensure that daily draws do not exceed 10% of an animal's circulating blood volume within a 2-week period. If at any point the maximal volume is met, we will not collect further samples until that 2-week period is over.
Blood for genetics: Blood may also be taken for the purposes of pharmacogenomics studies in collaboration with [redacted]. For the purposes of pharmacogenomics, up to two samples per NHP, a minimum of two weeks apart. Only one sample will be taken in the majority of cases. A second blood sample may be taken if the first is contaminated or in some other way unusable.

Vaginal Discharge: Vaginal swabs may be collected for the duration the subject is in the study (i.e., our collection may be continuous and may occur with all female subjects in the approved protocol). Vaginal swabs may be collected on a daily basis to track different phases of the menstrual cycle. These samples may be used to correlate with progesterone and estradiol levels or may be used to track the menstrual cycle in the absence of progesterone and estradiol depending on the experimental endpoint.

5. Will the animal(s) be anesthetized or sedated during this procedure?
☒No ☒Yes

If No, describe restraint method. (Note: If methods require a prolonged period of restraint, Appendix G is required.)

Restraint will be limited to the squeeze-back mechanism on the cage. In our experience, well-trained monkeys may not require restraint at all, although the mechanism is always brought forward at least halfway as a safety consideration.

Blood samples will typically be taken without anesthesia for tracking of menstrual cycles and hormones, however, if an animal is sedated for reasons already described in the protocol (i.e., to fix a catheter issue, health check, etc.) on the same day the blood is needed, or if blood is being drawn for pharmacogenomics, the blood may be taken while the animal is sedated.

If Yes, list agents used for anesthesia and anaglesia:

Agent	Dose	Route	Frequency/Duration	Pharmaceutical Grade

Ketamine	10-20 mg/kg	i.m.	Once/sample	☒Yes ☐No
				☐Yes ☐No
				☐Yes ☐No
				☐Yes ☐No

For non-pharmaceutical-grade compounds:

a. **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

N/A

b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

N/A

Appendix F Administration of Drugs/Test Compounds

For instructions for completing this form, click here.

All agents given to the animals **must** be listed in this section with the exception of veterinary pharmaceuticals (antibiotics for treatment, anesthetics, and analgesics for treatment). Those will be listed in Appendix C.

NOTE: A pharmaceutical-grade compound (PGC) is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy.[1]
The Food and Drug Administration (FDA) maintains a database listing of FDA approved commercial formulations for both FDA approved human drugs (the Orange Book) and veterinary drugs (the Green Book).

Provide the following information:
*Note: all doses are mg/kg unless otherwise specified, all volumes are ml/kg unless otherwise specified.

Agent	Dose (mg/kg)	Volume (mL/kg)	Vehicle	Route	Frequency	NDC or CA	Hazard?	Pharmaceutical Grade
Alprazolam	0.01-10	0.01-0.1	Saline sterile H20 propylene glycol	i.v., i.m.	Once/day self-admin: max. 30 infusions/day	92623-85-3	Yes	☐Yes ☒No
Buprenorphine	0.01-1.0	≤ 0.2 ml/kg	Saline Sterile H20	i.v., i.m. s.c.	Self-admin (iv) 30 inf/day IM & SC: ≤6 injections/ d no more than 3 times/ week	53152-21-9	Yes	☒Yes ☐No
Cocaine Hcl	0.01-0.3 mg/kg/inj	0.001-0.1	Saline	i.v., i.m.	Once/day self-admin: max. 30 infusions/day	53-21-4	Yes	☐Yes ☒No
Clonazepam	0-10	0.1-1.0	Saline SterileH20 propylene glycol	i.v., i.m.	Max. 30 infusions/day no more than 2x/week	1622-61-3	Yes	☐Yes ☒No
CGS-9895 and other pyrazoloquinolones	1-30	0.01-1.0	Saline SterileH20 propylene glycol	i.v., i.m	Once/day	77779-50-1 (for CGS-9895)	Yes	☐Yes ☒No
Dexmedetomidine	0.03-0.06	< 1 ml/kg	--	i.m.	As an anesthetic **in combination with midazolam 0.3 mg/kg** prior to exams, blood draws,	17478-0055	Yes	☒Yes ☐No

					surgeries, etc. for ketamine-resistant NHPs			
Diazepam	0.1-10	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m.	Once/day self-admin: max. 30 infusions/day	439-14-5	Yes	☐Yes ☒No
D-cycloserine	1-10	0.01-0.1	Sterile H20	i.v., i.m	Once/day	68-41-7	No	☐Yes ☒No
Ethanol	0.1-3.0 g/kg	~5	Sterile H20	i.v.	No more than twice/week	62991-1663-01	Yes	☒Yes ☐No
Experimental GABA-A/benzodiazepine agonists (e.g., QH-ii-066; NE-594; HZ-166; JY-XHe-053; YT-III-31; PWZ-029)	Doses ranges for all compounds are 0.1-30 mg/kg	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	N/A	Yes	☐Yes ☒No
Experimental GABA-A/benzodiazepine antagonists (e.g., XLi-093; BCCT; 3-PBC; DM-D-053)	Dose ranges for all compounds are 0.3 – 30 mg/kg	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	N/A	Yes	☐Yes ☒No
Experimental GABA-A/benzodiazepine inverse agonists (e.g., RY-23), PWZ-029 (mixed agonist/inverse agonist)	RY: 0.03-1.0 PWZ: 0.001-1.0	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	N/A	Yes	☐Yes ☒No
Flumazenil	0.01-10	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day	78755-81-4	No	☐Yes ☒No
Finasteride	0.001-0.1	0.1-1.0	Saline	i.v., i.m.	No more than twice per week	98319-26-7	Yes	☐Yes ☒No
Fentanyl	0.00001-0.1 mg/kg/injection	0.001-0.1	Saline SterileH20	i.v.	Self-admin: max. 30 inf/day	990-73-8	Yes	☒Yes ☐No
Heparin		100-150 units per ml	Saline	i.v.	≤6 injections/ day (used to flush drug and maintain catheter patency)	63323-540-11	Yes	☐Yes ☒No
Heroin	0.0001-0.1	0.01-0.1	Saline	i.v.	Self-admin: max. 30 inf/day	561-27-3	Yes	☒Yes ☐No

Histamine	0.0001-0.1	0.01-0.1	Saline	i.v.	Self-admin: max. 30 inf/day	56-92-8	Yes	☐Yes ☒No
Ketamine	0.3-20	≤ 0.2 ml/kg	Sterile water	i.v., i.m.	As an anesthetic prior to exams, blood draws, surgeries, etc. maximum 3x/week unless consult w/vets approves up to 5x/week	0856-2013-01	Yes	☒Yes ☐No
L-838,417	0.3-3	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	286456-42-6	No	☐Yes ☒No
L-655,708	0.1-3	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	130477-52-0	No	☐Yes ☒No
Methadone	0.01-10.0	≤ 0.2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv) 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	1095-90-5	Yes	☐Yes ☒No
MRK 016	1-30	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m	Once/day Self-admin: max. 30 inf/day	783331-24-8	No	☐Yes ☒No
Midazolam Hcl	0.1-1.0 mg/kg/inf	0.01-1.0	Saline	i.v., i.m.	Once/day Self-admin: max. 30 inf/day	00409-2308-01	Yes	☐Yes ☒No
Midazolam Hcl	0.3	< 1ml/kg	-	i.m.	As an anesthetic **in combination with dexmedetomidine 0.03-0.06 mg/kg** prior to exams, blood draws, surgeries, etc. for ketamine-resistant NHPs	00409-2308-01	Yes	☐Yes ☒No
Midazolam Maleate	0.1-1.0 mg/kg/inf	0.01-1.0	Saline	i.v., i.m.	Once/day Self-admin: max. 30 inf/day	59467-94-6	Yes	☐Yes ☒No
Morphine	0-5.6 mg/kg/inf	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day	64-31-3	No	☒Yes ☐No

					IM & SC: ≤6 injections/ day, no more than 3 times/ week			
Methohexital	0-3 mg/kg	≤ 2 ml/kg	Saline	IV	Up to once/day	18652-93-2; 151-83-7	Yes	☒Yes ☐No
Methampheta mine Hcl	0.001-1.0 mg/kg/inf	0.001-0.1	Saline	i.v., i.m.	Self-admin (iv): max 40 inf/day IM & SC: ≤6 injections/ day, no more than 5 times/ week	51-57-0	Yes	☐Yes ☒No
Neuroactive steroids, including Ganaxolone, progesterone, & Pregnanolone	0.001-100	0.1-1.0	Saline SterileH20 propylene glycol, β-cyclodextr in	i.v., i.m.	Once/day Self-admin: max. 30 inf/day (PR) max. 150 inf/day (BE)	38398-32-2 57-83-0 128-20-1	Yes	☐Yes ☒No
Nalfurafine	0-0.1 mg/kg/inf	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	152658-17-8	Yes	☐Yes ☒No
Naloxone	0-2 mg/kg/inf	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	119630-94-3	No	☐Yes ☒No
Naltrexone	0-2 mg.kg/inf	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	16676-29-2	No	☒Yes ☐No
Nor-binaltorphimin e	0-5.6 mg/kg/inf	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	105618-26-6	No	☐Yes ☒No
Orexin Receptor antagonists (e.g., non-selectives: almorexant,	0.1-100	≤ 0.2 ml/kg	Saline, SterileH20 , Propylene Glycol, β-	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more		Yes	☐Yes ☒No

suvorexant; subtype selectives: SB-334867, JNJ-42847922)			cyclodextrin		than 5 times/ week			
Oxycodone	0-0.3	≤ 2 ml/kg	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	64-31-3	Yes	☐Yes ☒No
Remifentanil	0.00001-0.001 mg/kg/inf	0.001-0.1	Saline SterileH20	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	132539-07-2	Yes	☐Yes ☒No
Salvanorin A	0-0.1 mg/kg/inf	≤ 2 ml/kg	SterileH20 Ethanol & Tween 80	i.v., i.m., s.c.	Self-admin (iv): max 30 inf/day IM & SC: ≤6 injections/ day, no more than 3 times/ week	83729-01-5	Yes	☐Yes ☒No
Scopolamine HBr	0.003-0.3	≤ 2 ml/kg	Saline	i.m., i.v.	No more than 2x/week	6533-68-2	Yes	☐Yes ☒No
Stimulants (e.g., d-amphetamine, MDMA, methylphenidate)	0-1.0 mg/kg/inf	0.001-0.1	Primarily Saline, may use SterileH20 or Propylene Glycol	i.v., i.m., s.c., p.o.	Self-administered: Determined by animal, typically ≤40 injections/day Experimenter administered: ≤6 injections/day ≤5 times/week		Yes	☐Yes ☒No
Triazolam	0.001-1	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m.	Once/day Self-admin: max. 30 inf/day (PR), max. 150 inf/day (BE)	28911-01-5	Yes	☐Yes ☒No
TP 003	0.01-30	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m.	Once/day Self-admin: max. 30 inf/day	628690-75-5	No	☐Yes ☒No
TB 21007	0.1-10	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m.	Once/day	207306-50-1	No	☐Yes ☒No
Temazepam	0-3.0 mg/kg/inf	0.001-0.1	Saline SterileH20 propylene	i.v., i.m.,	Once/day	63739-877	Yes	☐Yes ☒No

			glycol	s.c., p.o.	Self-admin: max. 30 inf/day			
Zolpidem	0.1-30	0.01-0.1	Saline SterileH20 propylene glycol	i.v., i.m.	Once/day Once/day Self-admin: max. 30 inf/day	82626-48-0	Yes	☐Yes ☒No
Vehicles								
0.9% sterile saline	Up to 100% of solution	≤ 2 ml/kg	-	IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week		No	☐Yes ☒No
Ethanol	≤ 20% of a solution	≤ 2 ml/kg		IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week	62291-1663-01	No	☒Yes ☐No
Tween 80	≤ 10% of a solution	≤ 2 ml/kg		IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week	9005-65-6	No	☐Yes ☒No
Sterile Water	Up to 100% of solution	≤ 2 ml/kg		IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week	-	No	☐Yes ☒No
Propylene Glycol	Up to 80% of solution	≤ 2 ml/kg		IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week	57-55-6	No	☐Yes ☒No
β-cyclodextrin (hydroxypopyl -beta-cyclodextrin 97%)	Up to 50% of solution	≤ 2 ml/kg		IV, IM, SC	IV: ≤30 injections/ day IM & SC: ≤6 injections/ day, no more than 3 times/ week	CAS 128446-35-5	No	☐Yes ☒No

NDC# is preferred over CAS#, if available. The NDC# will be on the bottle or box if the substance is a pharmaceutical. If there is no NDC# then include the CAS#. CAS# and hazard information can be obtained from the MSDS sheet through the UMMC Intranet (http://www.umc.edu/intranet/index.php). Choose the

"MSDS On-Line" link under "Hot Spots".

1. Describe any potential adverse side effects that may result in the animal from the administration of this material. If agents are unknown or their potential side effects are not documented, provide a reasonable estimate of the effects of the general class of chemicals (e.g., compound may have sedative properties, compound will likely produce diarrhea, etc.).

Some of the drugs/compounds in this protocol can result in physical dependence with associated withdrawal syndrome. For these studies, however, physical dependence would be a confounding factor—it is not a planned experimental endpoint. Because of this, as well as the safety of the monkeys in general, all experimental conditions are such that the likelihood of dependence is extremely low. For all studies, monkeys will be observed by trained staff immediately after the experimental sessions for sedation, ataxia and/or withdrawal signs (tremors, retching, vomiting). In the unlikely event that mild withdrawal-like indications develop, diazepam (1 – 3 mg/kg, b.i.d., i.m., i.v., or to effect) will be administered immediately to alleviate the physical symptoms, and the experiment will be ended by gradually reducing the availability and/or drug dosage over a period of weeks to avoid possible precipitation of more severe symptoms. Pharmaceutical-grade diazepam doses may not be high enough to alleviate withdrawal symptoms. For this reason, non-pharmaceutical grade diazepam is mixed in the appropriate concentrations to have the intended effect.

GABA-A/benzodiazepine agonists (e.g., diazepam, triazolam, etc.) could cause temporary and mild sedation and/or ataxia. If deemed hazardous, these effects are reversible with administration of the antagonist flumazenil (0.3 – 3 mg/kg, i.m. or to effect).

At doses above those proposed for use in our studies, GABA-A/benzodiazepine inverse agonists can be proconvulsant. Animals will be observed routinely by laboratory staff during and after the experimental session for any signs of convulsant activity. These effects are reversible with administration of diazepam (1 – 3 mg/kg, b.i.d., i.m., or to effect).

Ketamine and remifentanil will be administered at doses and concentrations, and by routes of administration, within proven safe parameters in monkeys and are not expected to produce any adverse side effects that would require medical attention or endanger the health of the animal.

Heroin and fentanyl can induce physical dependence following long-term treatment of relatively intermediate-to-high doses. For our studies, however, physical dependence would be a significant confounding factor – it is not a planned experimental endpoint. Because of this, as well as the safety of the monkeys in general, all experimental conditions are such that the likelihood of dependence is extremely low. For all studies, monkeys will be observed by trained staff immediately after the experimental sessions for sedation, ataxia and/or withdrawal signs (vomiting, retching, tremors). In the unlikely event that mild withdrawal-like indications develop, diazepam (1-3 mg/kg, b.i.d., i.m., or to effect) will be administered immediately to alleviate the physical symptoms, and the experiment will be ended by gradually reducing the availability and/or drug dosage over a period of weeks to avoid possible precipitation of more severe symptoms.

For Oxycodone, morphine, and remifentanil, no adverse events are expected at these doses, frequencies, or routes of administration. However, if side effects occur, they would likely include constipation, ataxia, sedation, and at high doses, respiratory depression. Naloxone or naltrexone will be administered I.V. or I.M. for suspected opioid overdose.

For histamine, the side effect of concern is appetite suppression, which can be alleviated by appropriate adjustment of food type and allotment, in consultation with CCR veterinarians. Weights of all monkeys are monitored carefully, and depending on the study can be obtained from once/month to as frequently as once/week (in most cases we obtain weights every two weeks). It is important to note that this side effect is quite rare based on the dosages proposed here.

Stimulants can suppress appetite, and at large doses, can induce seizures. Appetite suppression and/or weight loss can be alleviated by appropriate adjustment of food type and allotment, in consultation with CCR veterinarians. Weights of all monkeys are monitored carefully, and can be obtained as frequently as necessary while the monkeys are in the chair. Monkeys will be given a time out period if 10 –15 % weight loss or decrease in body condition scoring is noted. It is important to note that this side effect is quite rare based on the dosages proposed here.
If seizures occur, diazepam (1-3 mg/kg or to effect, i.m. or i.v.) or midazolam (0.3-1 mg/kg or to effect, i.m. or i.v.), as approved in the original protocol, will be readily available so these drugs can be delivered as quickly as possible. Diazepam or midazolam will be stored in solution in a locked locker in close proximity to the animals so that the drug can be delivered as quickly as possible. The locked locker is accessible by research personnel who have been trained in the usage of diazepam and midazolam.
In the case of adverse effects, a CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

In the case of adverse effects, a CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

2. For each hazardous material, a Hazard Use form must be completed and attached it to the protocol. How many Hazard Use forms are included?

We list drugs and test compounds in our protocols as hazardous due to the fact that they may be toxic or hazardous at extremely high doses, but they are not hazardous or toxic at the concentrations used in our protocols. No appreciable amounts will be present in bedding, and no non-standard husbandry practices are required. Therefore, we have not filled out the attached hazard form(s) as they do not provide any additional useful information on the compounds outlined above.

<Link to Hazard Use form>

3. For non-pharmaceutical-grade compounds:
    a. **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

The goal of our research is to characterize drugs in self-administration, observation, and their effects on sleep parameters. Thus, our studies require formulations compatible for i.v./i.m. administration. This requires that, whenever possible, we administer pure forms of the compounds for which accurate dosages can be calculated. All non-pharmaceutical-grade compounds are either supplied from the National Institute of Drug Abuse drug supply program, purchased from vendors, or prepared by medicinal chemists.

*Note: A number of drugs are obtained as pharmaceutical-grade compounds, but are listed herein as non-pharmaceutical grade due to the fact that we dilute with various vehicles in

order to achieve the desired drug dose. Drug that are not diluted and administered from the purchased vial (e.g., ketamine, methohexital) are listed as pharmaceutical grade.

b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

We strive to acquire compounds with ~99% purity from commercial sources or our academic collaborator, prepare the solutions with precision instruments (and with trained personnel), and filter-sterilize all solutions with 200 µm filters into autoclaved containers.

Reference: UMMC Chemical Safety Manual
http://ehs.umc.edu/documents/ChemicalSafetyPolicy2010.pdf

Please remember that the use of any hazardous material in animal rooms requires that a sign be posted in that room and on the cages containing the hazard in accordance with the policy on Signage for Hazardous Studies.

[1] AAALAC Frequently asked questions about Non-Pharmaceutical Grade Compounds

Appendix G Prolonged Physical Restraint

Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation. Examples of prolonged physical restraint include: chairing of nonhuman primates, chronic harness restraint of metabolic animals, and tube restraints for rodents. For additional information, consult the IACUC's policy statement on Prolonged Physical Restraint.

1. **Justify** the need for prolonged physical restraint.

Self-administration, observation and EEG studies are conducted in the home cage. In order to administer investigational drugs, the animals are implanted with a chronic indwelling intravenous catheter. In order to protect the catheter, we must employ a vest and tether system (Lomir, Inc). Importantly, from the animal perspective, we use a 4 ft flexible stainless steel tether. This allows the monkey full range of motion to all aspects of the cage while still protecting the catheter, but is not long enough to pose danger to the animal. The vest is non-allergic nylon and well-tolerated by the monkeys. From the human perspective, intravenous administration of investigational drugs/compounds can be performed from outside of the cage and away from the monkey, reducing the potential for scratches and other injuries.

Conflict, tail dip, multiple sleep latency testing (MSLT), certain self-administration (port) studies and cognitive/behavioral procedures (for more information on procedures, see Appendix L) will be conducted in a remote experimental chamber. Monkeys will be brought to test chambers daily via primate restraint chairs. We chose to use the restraint chair for several reasons. First, our conflict procedure requires immobilization of the feet, and the safest method for doing this is to modify the chair, allowing for the accommodation of a shelf to hold the device used to administer the delivery of response-produced electric shock. Second, an experimental chamber provides precise stimulus control, which greatly enhances the ability of the animal to learn the task. This is especially important for MSLT, where sound and light attenuation are imperative. The conflict procedure, and especially our cognitive tasks, engages the ability of the monkey to use cues and temporal stimuli to successfully complete the task, which is not the case for self-administration and observation tasks, which are inherently simpler to perform.

2. Describe the restraint device.

All monkeys wear nylon vests while catheterized. For home cage-based studies, a 4-ft stainless steel tether will be attached to the vest and to a swivel device on the side of the cage.
Restraint chairs are custom made (e.g., Crist Instruments), and consist of a clear box and collar system that allows the monkey to be secure but sitting or crouching in a natural way that is species-specific to macaque monkeys. The box enclosure is open in the front for the monkey to manipulate levers and touch screens. The back of the enclosure consists of a door that is locked while the monkey is transported and serves as the point of entry for the monkey to and from the home cage.

3. Describe the details of how the animal(s) will be adapted to the restraint device.

Acclimation to jacket and tethers in the home-cage system occurs over a period of approximately one month. We proceed in stages – first allowing the monkey to habituate to the jacket and then adding the tether later (for the monkeys requiring the tether system).

Acclimation to primate restraint chairs occurs over a period of approximately 4 to 6 months, and is conducted by the PI and/or PI's staff. The acclimation process occurs in stages and monkeys' behavior and overall health and well-being are closely monitored. First, monkeys become habituated to a flexible stainless steel collar, a nylon collar, or a plastic collar (e.g., Primate Products). Alternative cloth and plastic collars are available in the instance that an animal may not readily habituate to the stainless steel collar. Next, the monkeys are trained to accept a metal chain leash attached to the collar or a Primate Products-style pole attached to the collar while remaining in the home cage. Once habituated to this procedure, a stainless steel custom-made pole is simply introduced into the home cage (unless the Primate Products-style pole is used), allowing the animal to become acclimated to the pole/leash system. Next, the experimenter will guide the animal from the home cage to the primate restraint chair and allow the monkey to accept a loose restraint at the waist and neck. To minimize discomfort, restraint chairs include adjustable perch bars and are custom-made in varying sizes to accommodate a range of animals.

The method of step-by-step habituation (referred to as "shaping by successive approximation") is intended to minimize distress and ensure safety for both the monkey and research staff. Throughout the process, animals are trained using positive reinforcement techniques, using palatable food rewards.

4. a. What is the duration of a restraint period?

Self-administration and observation studies are conducted in the home cage, and the cages have been modified to accept the swivel end of the tether. Thus, the animals are tethered 24 hr/day. Animals involved in conflict, tail dip, certain self-administration (port) studies and cognition procedures are trained to enter restraint chairs from the home cage. Monkeys will remain in chairs in remote test chambers for the duration of test sessions (test sessions not to exceed 3 hours per day). Post-test session, animals will be returned to the home cage.
For MSLT, monkeys will be chaired and brought to the test chambers no more than 3 times per week.

b. How frequently will an animal receive the restraint (e.g., daily, once per week, every month)?

24 hrs/day for the home cage system; up to daily (5x/week) for the chair system.

5. Are animals monitored during the restraint period? ☐No ☒Yes
How often?

Animals are monitored on a daily basis by laboratory staff. On days on which the

experiment is conducted (typically Monday through Friday, excluding holidays), animals are observed a minimum of four times/day.

6. Are there any anticipated problems as a result of the restraint device (e.g., skin lesion from harness, moist dermatitis, etc.)?

There is the possibility of skin lesions developing under the vest (e.g., in the shoulder or belly area). We minimize this possibility by adjusting the vest at the neck and belly to be as loose as possible, yet still protect the catheter. In extreme instances, we have had custom vests made for monkeys by Lomir. Monkeys will be checked for lesions by laboratory staff every time they are sedated or in a restraint chair; minimally every two weeks coinciding with cage change out. When checking the monkey in a restraint chair, the area that can be observed is often limited to the back; therefore these monkeys are also sedated approximately every two weeks to one month for a more complete examination.

Appendix H Multiple Survival Surgical Procedures

A major surgical procedure is defined as a surgical intervention that penetrates or exposes a body cavity (peritoneal, thoracic, cranium), produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Guide, 2011). Multiple procedures are those whereby an animal will regain consciousness after each procedure. Procedures must be described in Appendix C. A surgery followed by a second procedure where the animal is euthanized is not considered multiple surgical procedures.

Surgeries performed on the animal prior to the animal's arrival at UMMC (e.g., ovariectomy procedure performed by vendor) must be considered. For additional information consult the IACUC's policy statement on Multiple Major Surgical Procedures.

1. **Justify** the need for multiple major surgical events in a single animal.

Catheter surgeries:
NHPs have up to 8 vessels (2 internal jugular veins, 2 external jugular veins, 2 brachial veins, and 2 femoral veins) that may be catheterized, depending on the disposition of the monkey. Surgery (as described in appendix C) is required to implant a catheter in each of these veins.

Sometimes a catheter may break at the site of the exit from the back and will, in the majority of cases, re-enter the body. In these cases it is necessary to make a small incision to re-expose and re-route the catheter back out the exit site (as described in Appendix C). Surgery is also required to remove or replace catheters that have become occluded or dislodged. For NHPs with subcutaneous ports, there is a possibility that the port could become unanchored or infected, in which case a surgery would be required to re-anchor or replace the port.

EEG/Telemetry Surgeries:
Implants will remain in the monkey until the batteries die out, which is expected to take approximately 6-12 months (see appendix C), after which a surgery can be performed to replace the implant with a new one while leaving previously placed leads intact. Surgery may also be required if signals from leads are no longer good and the lead must be replaced. Decisions about implantation of new leads will be made in consultation with DSI and CCR vet staff. Leads will be replaced a maximum of one time, and if the second lead fails, the NHP will be re-assigned to experiments not requiring telemetry devices.

2. What is the time interval between the surgical events?

A minimum of 1 week recovery period is required between all surgeries.

Catheters are expected to remain patent for 6 to 36 months.

Appendix I Food and/or Fluid Regulation

The Guide (2011) states: "Regulation of food or fluid intake may be required for the conduct of some… research protocols. The regulation process may entail ***scheduled access*** to food or fluid sources, so animal consumes as much as desired at regular intervals, or ***restriction***, in which the volume of food or fluid consumed is strictly monitored and controlled." The least restriction necessary to achieve scientific objectives while maintaining animal well-being should be used. For additional information consult the IACUC's policy statement on Food and/or Fluid Regulation.

1. Will ☒FOOD or ☐FLUIDS be ☒**scheduled** or ☒**restricted**?

Justify the need to schedule or restrict food and/or fluid.

In experiments involving food delivery, stable daily performance is maintained by restricting access to monkey chow in the animal's living quarters. Performance is maintained by commercially-available food pellets which are flavored (e.g., banana, fruit punch) and nutritionally balanced, which we have found to provide the best level of motivation for our studies while allowing us to maintain a relatively mild degree of food restriction. During initial training, body weights are reduced to approximately 90% of free-feeding values. Once subjects respond reliably under the schedule of food delivery, home cage food availability is increased to the maximum allotment that can be given without resulting in degraded performance during experimental sessions. Fruit, vegetables, and vitamins are given as supplements at least twice weekly. Over the course of the experiments, we anticipate that body weights can be maintained at 85-95% of free-feeding values.

Rhesus monkeys can be maintained indefinitely at such weights with no untoward effects or risks to health. Calorie restriction up to 30% has been shown to increase life expectancy in certain species of laboratory animals and to reduce the incidence of type 2 diabetes, endometriosis, cardiovascular disease, and other diseases of aging in rhesus macaques (Kemnitz et al, ILAR Journal, 2011). As a result, we do not expect any significant negative health effects from the relatively mild restriction described here.

Food scheduling is typically carried out to maintain health of the monkeys. Weight gain and obesity can have profound effects on the disposition of benzodiazepines, which are characteristically highly lipophilic. We also wish to keep weights to appropriate levels due to the unknown consequences of rhesus monkeys having chronic venous catheters but experiencing cardiovascular events due to obesity.

2. Check all methods that will be used to ensure adequate nutritional intake and hydration.

METHOD		FREQENCY OF CHECKS
Body weight	☒	At least monthly; typically bi-weekly
Urine output	☐	
Fecal output	☐	
BUN	☐	
Hct	☐	
Food intake	☒	Daily
Other	☒	Physical exam, BCS & somatometric measurements at least monthly

3. Restriction protocols typically base the restriction amount relative to a baseline, (free-choice consumption) parameter (body weight, intake amount). What will this restriction amount use as the baseline?

The majority of monkeys assigned to this protocol were transferred to UMMC from the New England Primate Research Center (NEPRC). At NEPRC, they were ad lib fed for approximately 5-6 months before arriving at UMMC. After the habituation and quarantine period upon arriving at UMMC, weights were obtained and used as baseline. For monkeys acquired from other sources, a similar method will be used in which weights are obtained at the end of quarantine and used as baseline.

What is the maximum restriction for any animal?

85% of free-feeding weight

4. Growing animals must be frequently re-assessed to ensure normal growth patterns. If not using mature animals, what provisions will be made for these animals to assure that their nutritional needs are maintained?

There is a large range of ages represented in our monkey colony. In order to ensure the health and wellbeing of each monkey, we use body condition scoring and somatometric measurements (measurements in cm around the waist, sternum, and head-to-rump) in addition to weight (kg) to assess individual changes over time.

5. Describe the protocol for regulating food and/or water intake.

Food: Initial training of food-reinforced behavior and maintenance of stable daily performance is facilitated by restricting access to monkey chow in the animal's living quarters. During initial training, body weights are reduced to 85 - 90% of ad libitum values by gradually reducing the number of daily chow. Once subjects respond

reliably under the schedule of food delivery, home-cage food availability is increased gradually to the maximum allotment that can be given without resulting in degraded performances during experimental sessions. Fruit, vegetables and other food-based enrichment items are given as supplements. We will monitor weights regularly to ensure that animals remain in the desired window. Monkeys will be weighed every 2 weeks during cage change to limit risk to personnel. Body weights are not permitted to fall below 85% ad libitum values. Amount of food is adjusted as necessary to maintain appropriate body weights. On days on which experiments are conducted, animals are observed at least four times daily by trained laboratory personnel.

6. How long will animals be on the regulation protocol?

Indefinitely; for the duration of their participation in the study. However, actual restriction level (85-95%) will depend upon the animal

7. Will animals have any access to unrestricted food or water at any time?

Rarely-- it is likely that food will continue to be restricted for the duration of the study. The actual restriction level will depend upon the animal. Unrestricted feeding will occur only in the event of a monkey not being in a study for an extended period of time.

Fluid will be available ad lib for monkeys in the home cage.

8. Who will be responsible for administering and documenting the regulation?

Personnel on this protocol (administering/documenting) and personnel of CCR (documenting, if appropriate).

Note: NPO procedures for pre-surgical fasting are not included in this consideration. NPO procedures shall not extend for greater than 24 hours; if surgical delays are encountered, the animals should be fed and re-fasted prior to the next scheduled procedure.

Appendix K Progress Report

1. Give a brief description of the work performed on these projects in the past 3 years. If progress did not occur or was less than expected, please give a brief explanation.

We have continued our research on the sedative and reinforcing effects of benzodiazepine drugs during the past 3 years, and are aiming to begin a number of new projects in addition to completing some already in progress (observation, self-administration, telemetry, etc.). During the past 3 years, we received new funding in the area of sleep/EEG, as well as a supplement to DA011792 under the HEAL initiative.

2. List any publications, abstracts, and/or presentations coming directly from the work performed on these projects in the past 3 years.

[redacted] Antagonism of triazolam self-administration in rhesus monkeys responding under a progressive-ratio schedule: In vivo apparent pA_2 analysis. *Drug Alcohol Depend* 158, 22-9, 2016

[redacted], Self-administration of progesterone and synthetic neuroactive steroids by male rhesus monkeys, *Drug Alcohol Depend* 165, 265-9, 2016

[redacted]; Choice between variable and fixed cocaine injections in male rhesus monkeys. *Psychopharmacology* 234, 2353-2364, 2017.

[redacted] Evidence that sedative effects of benzodiazepines involve unexpected $GABA_A$ receptor subtypes: quantitative observation studies in rhesus monkeys. *J Pharmacol Exp Ther* 366, 145-157, 2018.

[redacted]; Self-administration of benzodiazepine and cocaine combinations by male and female rhesus monkeys in a choice procedure: Role of α1 subunit-containing $GABA_A$ receptors. *Psychopharmacology*, in press, 2019.

[redacted] Evaluation of the anxiolytic-like, reinforcing, and sedative effects of YT-III-31, a ligand functionally selective for α3 subunit-containing $GABA_A$ receptors. *J Psychopharmacol*, accepted 2019.

3. Answer the following questions in regard to the last year of the previous version of this protocol.

I. Animals

1. Have any unanticipated (morbidity, mortality, inability to collect data) events occurred in the past year?

☐ Yes ☒ No

2. Has any mortality occurred prior to the anticipated end-point of an experiment or as a result of surgical manipulation?
   ☐ Yes ☒ No
3. Have any animals been euthanized prior to the anticipated end-point of an experiment?
   ☐ Yes ☒ No
4. Did any animals show signs of morbidity or sickness following experimental manipulation other than what was detailed in the protocol?
   ☐ Yes ☒ No

If yes to 1 -4, answer #5.

5. Describe any unanticipated events (morbidity, mortality, inability to collect data) and any identified contributing factors (e.g., recurring postoperative complications, excessive or unanticipated mortality rate, unplanned event that causes the removal of an animal(s) from an experiment for a period of time, loss of implant, etc.).

N/A

If the protocol involves breeding:

Breeding: Animals born over the past year as part of this protocol

Species	Strain	# of pups born in last year	# of pups used in the last year for experiments

What was the final disposition of any pups not used for experiments?

N/A

II. Personnel

1. During the past year did any Occupational Health & Safety "incidents or accidents" *(needle sticks, animal bites, cuts, burns, etc.) occur that involved personnel participating* in the conduct of this study?
   ☐ Yes ☒ No

2. If yes, describe the event and identify any contributing factors:

N/A

3. What treatment measures were taken:

There have been no NHP exposure events within the past year, however, should one occur all lab members have been trained to follow UMMC's NHP biohazard exposure protocol, which involves scrubbing the exposed area for 15 minutes, drawing blood from both the individual who was exposed and the

animal to ensure that there is no presence of the Herpes B virus, and taking retroviral medication (e.g., Valtrex).

Appendix L Behavioral Training and Testing

Useful Resources:

NIH Publication: *Methods and Welfare Considerations in Behavioral Research with Animals NIH Publication No. 02-5083, March 2002*
http://www.nimh.nih.gov/researchfunding/animals.pdf

American Physiological Society Publication: *Resource Book for the Design of Animal Exercise Protocols*, Feb. 2006
http://www.the-aps.org/pa/action/exercise/book.pdf

1. What form(s) of behavioral training/testing will be used?

Conflict procedure; operant drug self-administration; observation procedure; menstrual cycle monitoring; cognitive testing; activity monitoring; tail-dip testing; multiple sleep latency test; daytime and nighttime EEG/EMG/EOG recording.

2. Describe how the behavioral training/test is conducted (include descriptions of the devices, preliminary animal training, fluid/food restriction, reward/ positive reinforcement, duration of trial, frequency of behavioral testing, etc.).

2a. Conflict procedure:
2a.i. Conflict procedure training: Prior to training, all monkeys are implanted with chronic i.v. catheters, attached or not to subcutaneous vascular access ports, as described in detail in Appendix C. Monkeys are first trained to sit in a restraint chair, and animals without subcutaneous vascular access ports are also habituated to wearing a custom-designed nylon jacket (see Appendix G for details).

BZs characteristically increase lever-press responding for food presentation that has been associated with delivery of a brief, mild electric shock. This is called an "anti-conflict" effect (i.e., anxiolysis) and represents a highly valid approach to discovering new anxiety-reducing agents (i.e., anxiolytics). In our version of this task, monkeys are trained to respond under a procedure that has two components. One component is associated with a distinctive visual stimulus (e.g., red light) and the second component is associated with a different stimulus (e.g., green light). In each component, completion of 1 or more responses will produce a food pellet followed by a brief "timeout" period during which pressing a lever will have no consequence. The component will end following delivery of 5 food pellets or 5 min, whichever occurs first. An extended timeout period (typically 5 - 30 min, depending on the time course of action for the particular drug in question) will proceed each of four cycles with two components (1 red, 1 green) per cycle.

When performance in both components of this procedure is stable, responding in one component will be suppressed by superimposing response-produced electric shock. Under the superimposed part of the session, every nth response (n=1 or more, adjusted for each monkey depending on individual performance) will produce shock. Shock intensity will be in the range of 1 - 3 mA for 0.25 sec, a level sufficient

to suppress behavior but below the pain threshold (Vierck et al. 1983). Moreover, suppression of behavior by this level and duration of shock is not sensitive to pain medication, such as morphine. Please note that this shock is entirely escapable via withholding responding, and in fact, most monkeys learn to press the lever enough to receive a food pellet but stop prior to any shock delivery.

2a.ii. Conflict procedure testing: Once responding is stable (at least three sessions in which suppressed responding is less than 50% of non-suppressed responding, with no upward or downward trend in response rates in -either component), drug testing will begin. During test sessions, BZs, BZ-type compounds and neuroactive steroids will be administered i.v., alone and in combination, during the extended timeout periods using a cumulative dosing technique. This technique permits determination of a four-point dose-response function in a single experimental session. In most cases, a broader range of doses will be studied by determining the effects of overlapping ranges of cumulative doses in separate sessions (e.g., 0.01 - 0.3 mg/kg during one test session and 0.1 - 3 mg/kg during another test session). Experiments in which overlapping doses are studied also permit assessment of the reproducibility of drug effects over the most relevant portion of the dose-response functions and enable comparison of the effects of particular doses that are administered at different points in the cumulative dosing sequence.

2b. Operant drug self-administration:

Prior to training, all monkeys are implanted with chronic i.v. catheters, attached or not to subcutaneous vascular access ports, as described in detail in Appendix C. Self-administration sessions can happen in the home-cage or in an operant chamber. Animals without subcutaneous vascular access ports are habituated to wearing a custom-designed nylon jacket, and for animals doing home-cage self-administration, the jacket is attached to a tether and swivel (see Appendix G for details).

For self-administration sessions happening in the home-cage, an operant panel will replace one of the sides of the home-cage. The operant panel contains stimulus lights and response levers, as well as a food pellet dispenser, which are controlled by a remote computer.

For self-administration sessions happening in the operant chamber, animals are also trained to sit in a restraint chair. For the duration of the self-administration session (both training and test sessions), animals are positioned in a primate chair (Crist Instruments, Inc.; Primate Products style-custom manufactured) and placed in a ventilated, sound-attenuating experimental chamber facing the operant panel located inside the chamber. The operant panel contains stimulus lights and response levers, as well as a food pellet dispenser, which are controlled by a remote computer. For experiments conducted in animals implanted with a subcutaneous vascular access port, once the monkey is inside the chamber, the access port area is prepared under aseptic conditions (hair is removed using clippers, and the site is prepared with three alternating applications of 70% alcohol and povidone/iodine prep solution), and a Huber needle (Access Technologies) is inserted into the access port. The design of the needle minimizes damage to the port membrane and

allows for repeated punctures over a year or more. The polyvinyl-chloride tubing attached to the Huber needle is connected to a motor-driven syringe located outside of the chamber containing the drug solution. For animals using jackets, a connecting pin and an extra catheter line will be used to attach the catheter to the motor-driven syringe. Computers control experimental events and record data (lever presses).

Monkeys initially are trained to respond by pressing a lever once to receive an i.v. injection of methamphetamine (0.001-1.0 mg/kg/injection), midazolam (0.01-0.1 mg/kg/injection), methohexital (0.003-0.1 mg/kg/injection), cocaine (0.03-0.3 mg/kg/injection), remifentanil (0.0001-0.001 mg/kg/injection), or fentanyl (0.00001-0.1 mg/kg/injection). Once the animal has acquired the lever press response and behavior is reliably maintained by drug injections, the response requirement can be gradually increased under a variety of intermittent schedules of reinforcement.

2.b.i Progressive-ratio (PR) schedule of reinforcement

Progressive-ratio training:

Once responding on the lever is engendered, the response requirement is gradually increased to the terminal value, which may vary depending on the subject and/or experimental condition. Once stable responding is established (± 20% variation over 3 days with no consistent trends), saline will be substituted for drug until responding is reduced to low levels (less than 5 injections/session) and again meets the stability criteria. Once a clear difference between self-administration of saline vs. drug is established, responding will be re-established with training drug and monkeys will be trained under a procedure referred to as progressive-ratio (PR) schedule. This procedure provides a quantitative means for determining how strong a reinforcer a drug is, as determined by a measure referred to as "breakpoint" (highest response requirement completed in a session). Daily experimental sessions will start with the illumination of stimulus lights and will consist of at least five components, each made up of individual trials (e.g., 20 trials total). Each trial within a component will have the same response requirement and will be separated by a timeout (30 min or longer if required) in order to minimize drug accumulation due to repeated injections in a single session.

Once a trial is initiated, completion of a specified response requirement will result in drug injection. A trial will end with the injection or the expiration of a limited hold, at which time the stimulus lights will be extinguished and the timeout started. A session will end when the response requirement is not met within the limited hold for two consecutive trials or when all available trials are completed. During training sessions, the initial response requirement will be 1, and will double across the components to a maximum of 16. This PR sequence will be increased to an initial response requirement of up to 40 per injection (i.e., response requirements of 40, 80, 160, 320, 640). In our experience, this sequence results in stable self-administration of BZs, and the response requirements are high enough to prevent self-administration of the maximum of 20 injections/session. However, if the maximum number of injections/session is consistently self-administered for a particular drug (i.e., a ceiling effect occurs), the sequence will be adjusted by increasing the response requirements (e.g., response requirements of 80, 160, 320, 640, 1280).

Progressive-ratio testing:
In our initial experiments ([redacted] et al. 2005; PNAS 102: 915-920), drug test sessions were conducted over multiple days of drug availability. We have developed a more rapid testing procedure in which sessions of drug and saline availability are alternated until a clear separation between self-administration of drug and saline is achieved. Using this rapid testing procedure, we have found that dose-response functions for drug self-administration under a PR schedule are similar to dose-response functions obtained using the more time-consuming method of our earlier studies. An additional advantage of this procedure is that the potential development of physical dependence is essentially eliminated, since a drug will be available for a maximum of 2 sessions in a row. In our planned experiments, sessions will alternate drug and saline according to the following 5-session cycles: DSSDD, SDDSS (S=saline, D=drug). Responding will be considered stable across the cycles when the number of injections/session are less than 5 for at least three sessions of saline availability and greater than 11 for at least three sessions of drug availability, with no consistent upward or downward trends. Test sessions will be inserted into the sequence every third session, e.g., DSTSDTD, STDDTSS, and so on (T=test). Each dose of test compound will be evaluated up to 2 times total. Compounds and doses will be tested in counterbalanced order, and all doses of a particular compound will be evaluated before another compound is tested.

2.b.ii. Fixed-ratio (FR) schedule of reinforcement
Fixed-ratio (FR) training:
Once responding on the lever is engendered, the response requirement is gradually increased to the terminal value, which may vary depending on the subject and/or experimental condition. Nonhuman primates rapidly acquire drug self-administration behavior under FR schedules, and stable daily performances can be obtained in several weeks. During self-administration sessions, the operant panel is illuminated with a stimulus light which serves as a discriminative stimulus. Completion of the FR results in a change in the stimulus light and a drug infusion. This infusion is followed by a timeout (60-s or longer if required). At the end of the timeout, the discriminative stimulus light is presented again to signal the opportunity to complete another FR. Drug intake is determined as the number of infusions received on a given session times the unit dose of drug. Total session intake of drug is a direct function of response rate under FR schedules. For specific behavioral experiments, once stable responding is established (±20% variation over 3 days with no consistent trends), animals can be submitted to extinction by substituting the drug with saline until responding is reduced to low levels (<20% of average maintenance response rates) and again meets the stability criteria. Extinction sessions can be followed by reinstatement sessions consisted of an experimenter-administered i.v. prime of the self-administered drug immediately before the onset of the session, with completion of the response requirement resulting in the intravenous delivery of saline. Drug intake will be limited by restricting the total number of drug injections per session or by restricting the duration of the self-administration sessions (sessions not to exceed 3 hours per day).

Fixed-ratio (FR) testing:
Before the beginning of drug-interaction studies, the unit dose of self-administered drug will be altered until the EDMax (the maximally effective behavioral-stimulant dose of drug) is identified for each individual subject. For drug-interaction studies, the effects of pretreatment with different compounds and doses will be tested in combination with different doses of self-administered drugs (EDMax and one-half log-step unit dose below and above the EDMax dose). Stable self-administration behavior will be defined as response rates that varied by <20% over 3 days. Once responding stabilizes at a given dose, different compounds and doses will be administered before the initiation of the self-administration or reinstatement sessions. Between different pretreatments, animals will be returned to normal drug self-administration or reinstatement conditions. Reinstatement sessions with vehicle pretreatment will alternate with reinstatement sessions with compounds pretreatments to ensure a reliable reinstatement response. If the reinstatement effect dissipates, animals will be returned to maintenance self-administration conditions until stability criteria are met again, then a new block of extinction and reinstatement will begin. The order of drugs, compounds and doses will be randomized and counterbalanced across subjects.

2.b.iii. Choice self-administration
Surgical procedures for these studies are the same as described in Appendix C, but animals will be implanted with catheters that have double lumens instead of single lumens.
Choice training:
Once responding on a single lever is engendered, animals will be trained to self-administer drug on one of two concurrently available levers. Each session will consist of "sampling" trials, followed by "choice" trials. For the sampling trials, one lever is active at a time, and the two options will be alternated, signaled by retractable levers and stimulus lights above the lever. The sampling trials ensure that the monkeys are exposed to the choice contingencies. Following the sampling trials, the choice trials occur with both levers available and all lights on.
Choice testing:
For all groups, the training drug alone will be available via one lumen, and the other lumen will contain the same dose of training drug mixed with one of several doses of a test compound. A given choice condition will be in effect until choice is stable, defined as (1) the number of choices of the drug alone is within 20% of the 3-session mean for 3 consecutive sessions and (2) there are no upward or downward trends over the 3 sessions. Once stability is achieved, the injections associated with the levers will be reversed (to assure stimulus control).

These studies in general will involve varying the amount of the training drug dose (without the test compound) until consistent self-administration at 50% choice is achieved (i.e., "indifference"). Next, we will conduct tests with known and novel reinforcing, punishing, and neutral drugs in order to demonstrate enhancement, attenuation, and neutral (no) effects, respectively. For these tests, the test drug will be mixed with one of the novel drugs.

2c. Observation Procedure:

All monkeys are implanted with chronic i.v. catheters, attached or not to subcutaneous vascular access ports, as described in detail in Appendix C. Animals are habituated to wearing a custom-designed nylon jacket, and the jacket is attached to a tether and swivel (see Appendix G for details). Behavioral profiles will be determined after monkeys habituate to the human observer (typically 1-2 weeks). After catheter implantation, baseline control data will be obtained following saline or vehicle injections.

The behavioral effects of each compound will be determined using quantitative observational techniques. The basic design is as follows: (1) Acute dose-response and time-response determinations for compounds alone; (2) tests of compounds in combination, based on the effects of the compounds alone. A range of species typical behaviors, as well as behaviors associated specifically with BZ administration, will be recorded by trained observers. All observers will be unaware of the goals of the study as well as the compound under investigation. Observers will be trained using a standard procedures manual and will complete at least 20 hrs of scoring prior to participating in the study. Reliability will be checked every 3 months to insure consistent data across observers and time. Using this scoring system, the presence of a behavior is noted during each 15-second interval and the number of 15-second intervals during a 5 minute session is observed is recorded. Thus, the maximum score for each behavior during one modified frequency sample is 20.

2c.i. Sedation measures: We have developed a scoring system for sedation based on standards used for anesthesia of human patients used by the American Society of Anesthesiologists (ASA 2002). Based on these standards, two categories are included in the behavior scoring session: Moderate sedation and deep sedation. These measures include an evaluation of responding to external stimuli during the modified frequency scoring session. The evaluation of responsiveness is conducted at the beginning of each 60-second block of time during the scoring session (i.e., maximum of 5 evaluations during one session). If a behavior referred to as "sleep/rest posture" is observed and the animal does not attend to the activities of other monkeys in the room, the observer will speak the monkey's name in a normal tone, walk at a normal pace towards the cage, and then move the lock used to secure the door of the cage (a feature present on all cages). The different types of response to external stimuli are described as "responds readily", "delayed response" or "no response". If the monkey opens its eyes and initiates movement in less than 3 sec, the observer will stop the evaluation and score "sleep posture". If the monkey attends slowly (i.e., 2 3 seconds following stimuli) the observer records "delayed response" which is part of a decision rule for "moderate sedation", whereas "no response" identifies "deep sedation". The interaction of these response-to-external stimuli criteria and the scoring of behavior allow for easier interpretation of atypical situations, e.g., a monkey in a normal sleep-associated posture but unresponsive would be scored as "deep sedation" based on the "no-response" criterion being met.

2d. Menstrual cycle monitoring:

2d.i. Training: Animals will be trained by the Pl's staff to undergo awake i.v. blood sampling (taken from a peripheral vein, e.g., saphenous vein, cephalic vein, etc.), as well as vaginal swabs, using positive reinforcement techniques. Specifically, the method of "successive approximations" will be used, in which components of the final task are rewarded such that the behavior is "built up" gradually. For example, a monkey first will be gently placed in the front of the cage using the squeeze-back mechanism, and rewarded with highly palatable food (e.g., apple slices, banana, raisins). Once the monkey accepts this manipulation, another approximation of the behavior will be introduced, such as presenting the leg via the cage door for i.v. sampling, or a swab placed near the vagina for vaginal swabbing. Once the monkey accepts the next approximation, rewards for the original component are omitted, with the goal of rewarding only the last component of the chain of events. Based on consultation with colleagues who are expert with these techniques, we anticipate about 3 months for i.v. sampling and 1 month for vaginal swabs.

2d.ii. Sampling procedures: For a given procedure described above, the behavioral task (i.e., conflict, self-administration, observation) will be halted in order to determine menstrual cycles under "baseline" conditions, i.e., with no other procedures going on. First, we will initiate training of daily vaginal swabs (once per day, 7 days per week) and i.v. sampling (daily manipulations, with an i.v. insertion occurring only after the animal calmly allows a leg to be handled and allows fur to be shaved on the calf without struggling). Once training is complete, we will take ~1-2 ml samples daily for approximately 1 month, i.e., from day 1 of a positive vaginal swab to the next positive vaginal swab. This volume of blood collected will not exceed 10% circulating blood volume every 2 weeks.

Daily sampling is necessary to conduct at least once in each monkey. This will allow us to precisely determine the time of the rise in estradiol levels and subsequent increase in progesterone. The progesterone increase typically occurs over 2-5 days by approximately day 25 post-positive swab, and in some cases does not occur. If no progesterone peak is observed, we will rest the monkey over one swab-to-swab interval, then sample daily again.

Behavioral testing will begin upon establishment of menstrual cycles. We will be able to rapidly determine estradiol and progesterone levels by use of the Oregon National Primate Research Center's fee-based service (or another appropriate service). We will evaluate test doses of BZ-type drugs starting at the first positive swab and stopping at the end of the interval (i.e., the next positive swab). To minimize the number of blood samples taken, we will collect the samples every 3rd or 4th day, based on the baseline sample determination. The first determination, however, will not have samples taken-if an increase is observed at the appropriate time (e.g., day ~25 post-positive swab, then the dose will be repeated and very targeted samples will be taken (e.g., daily for 3-5 days once behavior is elevated). In the event that no behavioral change occurs, sampling will occur at 1- 2 day intervals to (1) confirm progesterone peaks are occurring and if so (2) confirm that elevations in progesterone do not influence behavior.

Because of the frequency of blood samples, we will interact closely with the CCR veterinarians in order to monitor (1) the condition of the monkey's legs with repeated injections, and (2) red blood cell levels. Regarding the former, a clinical veterinarian

will be notified promptly if any complications are observed. Excessive bruising or other signs of trauma will be considered indicators that the sampling will be halted and re-initiated during the next positive swab-to-swab interval.

Regarding red blood cell levels, CCR staff will be consulted, and if needed, hematocrits will be taken once per week during the studies to insure that red blood cells are not depleted by the sampling procedures. Given the very low levels of blood taken, the extensive literature on this procedure, and consultation with the Division of Reproductive Biology of the Oregon National Primate Research Center, we do not anticipate any significant problems with regards to the animals' legs and overall health.

2e. Cognitive testing:

2e.i. Hands-on testing: Object Retrieval with Detours (ORD), Object Discrimination Reversal (RL), and Novel Object Recognition (NOR). Testing is conducted by trained technicians and occurs in the animal's home cage with the devices mounted to the front of the cage. Descriptions of the devices are provided with the specifics of each test. No preliminary training, food or fluid restriction is necessary to induce the animals to perform the tasks. Rather, positive reinforcement is used. In the ORD and RL tasks, monkeys are rewarded with food treats (e.g., marshmallows, life savers, fruit pieces); in the NOR task, the ability to interact with novel enrichment objects serves as positive reinforcement. For all three tasks, data collection can be accomplished in no more than 10 min/task. We typically opt to run two tasks/day and depending on the animals, it can take anywhere from 1 - 3 weeks to complete all phases of a given task. Assessment of performance will occur at 3 time points - before the animals begin drinking alcohol, after~ one-year of alcohol drinking, and after an extended period of abstinence.

<u>Object Retrieval with Detours (ORD):</u>

The device consists of a clear Plexiglas box with one open side that can be mounted to the cage front. The position of the open side can be varied (e.g., left, right). Food treats can be placed a varying locations in the open side (e.g., outside, inside, deep). Our dependent measures include the number of trials completed successfully as well as the type of error made.

Task phases:

Apparatus habituation I D – box in 'forward easy' position; food at edge of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row;

Apparatus habituation II D – box in 'forward easy' position; food at back of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row;

'Easy' training D – all easy trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row;

'Mixed' training D – mixed trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row;

Probe trial D – all difficult trials; 2 min to retrieve food; run one day.

<u>Object Discrimination Reversal (RL):</u>

The device consists of a tray with 3 recessed wells that can be mounted to the cage

front. The recessed wells can hold food treats and can either be uncovered or covered with specific objects. Our dependent measures include the number of trials completed successfully as well as the type of error made.
Task phases:
Apparatus habituation I – food in all 3 uncovered wells; 5 min to retrieve food; run until monkey retrieves all food within 5 min for 2 days in a row;
Apparatus habituation II – food in all 3 covered wells; 5 min to retrieve food; run until monkey retrieves all food within 5 min for 2 days in a row;
Acquisition – food under positive stimulus; allow monkey to 'find' treat; then 24 trials with position of positive stimulus varied; run until monkey retrieves 18/24 treats for 2 days in a row;
Reversal – 12 acquisition trials using previous positive stimulus; relocate/associate food reward with new object for 24 trials; run 24 reversal trials for 3 days in a row.

Novel Object Recognition (NOR):
The "device" is simply an array of typical enrichment objects hung on the front of the animal's home cage. Our dependent measure is the number of touches the monkey makes to the different objects.
Task phases:
Easy – two identical objects mounted 24 hrs/day for 4 days; one object replaced with a novel object on the test day;
Moderate – two different objects mounted 24 hrs/day for 4 days; one object replaced with a novel object on the test day;
Difficult – two different objects mounted 10 min/day for 4 days; one object replaced with a novel object on the test day.

2e.ii. CANTAB testing
CANTAB stands for Cambridge Neuroanatomical Test Automated Battery, and consists of a series of non-verbal cognitive tasks from the human CANTAB system. The CANTAB testing unit consists of a computer-controlled touch screen controlled by a remote computer. In addition to the screen, a pellet dispenser will be available for delivery of food pellets (Bio-Serv, 190 mg). After completion of the CANTAB tasks (approximately 1-2 hours), the monkey will be returned to the home cage and fed a daily allotment of food, adjusted for the food earned in the session, approximately 1 hour after the session.

Delayed non-matching to Sample (DNMS):
The DNMS task is a short-term recognition memory task involving sets of visual discriminations. A sample stimulus is presented in the center of the screen, and the animal must touch this stimulus within 30 second. After a touch, the screen is blanked and following a variable retention interval (0, 1, 3, 1O min, 1O min with distracter, which involves removing the monkey from the chamber for the delay period}, two stimuli are presented on the lower left and right of the screen. One stimulus is identical to the sample stimulus ("matching" stimulus) and the other is novel ("non-matching" stimulus). A touch directed to the non-matching stimulus is followed by reinforcer delivery. In addition to the four retention interval conditions, a

simultaneous condition is included in which the sample stimulus remains present while the matching and non-matching stimuli are present. A session consists of 10 trials of each retention interval and the simultaneous condition, presented in a randomly intermixed fashion (total trials=50). Performance accuracy is measured as the proportion of correct responses to all responses. The CANTAB software uses 469 shapes and 7 colors to ensure that discriminations are unique for approximately 120,000 trials.

Self-Ordered Spatial Search (SOSS):

The SOSS task is a short-term spatial working memory task similar to the radial-arm maze procedure used in rodents (Weed et al., 1999). In each trial, two, three or four small, colored, rectangular boxes are displayed on the screen in positions randomly allocated from 16 possible locations. The monkey must touch a box within 30 seconds of stimulus onset. After each successful touch, the color of the touched box is briefly (100 ms) changed and the screen is then blanked and a reinforcer delivered. After a 2-sec delay, the boxes are re-displayed and the monkey must touch a box which has not previously been touched in the trial in order to receive a food pellet. The trial is completed when the animal has either touched all boxes without a repetition (correct), touched a box that had previously been selected in that trial (error), or failed to touch a box within 30 sec of stimulus presentation (omission). Errors and omissions are followed by a tone and a 4-sec screen blank. After 5 sec, another trial is presented with stimuli in new (randomly allocated) positions. A session will consist of 40 trials grouped into 6 blocks that differ according to the number of boxes presented. The different blocks, with number of trials in parenthesis, will be: 2 boxes (5), 3 boxes (?), 4 boxes (?), 3 boxes (8), 4 boxes (8), 2 boxes (5).

Accuracy scores will be calculated for each trial type by dividing the number of correctly completed trials by the number of trials in which there was at least one response (omissions will be excluded from the calculation).

Intradimensional/extradimensional set shifting (ID/ED):

The ID/ED shift task evaluates the ability of a subject to attend to specific attributes of a stimulus as well as the ability to shift attention to other attributes when required. This task consists of a series of eight discrimination learning stages wherein touching only one of two stimuli presented on the screen results in food pellet delivery. Within any given stage of the task, a pair of stimuli is presented and the same stimulus is associated with reinforcement (S+ stimulus) until the performance criteria are met (18 of 20 consecutive trials correct). Correct choices must be made within 30 sec, and following a correct choice the screen is blanked for 5 sec while an incorrect choice (S- stimulus) results in a 0.2 sec tone and a 9-sec period of blank screen.

Eight distinct stimulus sets will be used. In the first stimulus set (stage 1), two distinctly shaped stimuli will be presented, with touches on one shape resulting in food pellet delivery. Stage 2 will consist of a stimulus reversal, in which the same two shapes are retained but pressing the S+ stimulus now does not result in reinforcement, whereas touching the former S- stimulus will result in food pellet

delivery. In Stage 3, a compound discrimination is presented. For this discrimination, the two shapes from the Stage 2 are present, but additional stimuli, consisting of lines, will be superimposed onto the existing shapes. Because the shape discrimination from the previous stage does not change, the lines are irrelevant to this discrimination. Stage 4 will consist of a shape reversal, similar to Stage 2. Stage 5 will consist of the intra-dimensional (ID) shift stage. For this discrimination, two new shapes with new lines will be presented. This is considered an ID shift due to the fact that despite new shapes and lines being introduced, the shape remains the relevant dimension for the discrimination. Stage 6 will consist of a reversal of the shape S+ and S- from Stage 5. In the final two stages, the extra-dimensional (ED) shift will be introduced. Thus, in Stage 7, new shapes and lines will be presented; however, the lines-not the shape-will be the relevant discriminative stimulus. Stage 8 will consist of a reversal of the line S+ and S- contingencies. Performance is determined as the number of errors at each stage, and the data will be subjected to a square root transformation to achieve normal distributions.

Paired associated learning (PAL):

Large colored abstract stimuli are displayed in one of 4 positions (top center, bottom center, left middle, right middle). The subject is required to touch the sample stimulus, which then disappears. After a 1-sec delay, the same pattern reappears (choice phase) in two or more locations on the screen (the original location plus one or more novel locations). The subject is required to touch the stimulus that is presented in the same location as the sample item to obtain a food pellet. Task difficulty is increased by increasing the number of stimulus-location associations required on each trial. Training is initiated with sessions that present 25 1-stimulus trials and 25 2-stimulus trials until performance averages >50% correct trials on the 2-stimulus trials. Next, monkeys receive sessions of 25 2-stimulus trials and 25 3-stimulus trials until performance averages >25% correct trials on the 3-stimulus trials. This part of the PAL task measures memory for stimulus-location associations. Next, a learning component is introduced in which a trial repeats if a mistake is made in attempting to complete this trial (i.e. the monkey gets 0-2 of 3 stimulus- locations correct in a 3-stimulus trial). Up to 6 attempts at a given trial are allowed. Finally, 4-stimulus trials are added when performance of 3-stimulus trials exceeds 50% correct. Thus, the PAL measures both memory and learning of stimulus- location associations.

Progressive-Ratio (PR) schedule of reinforcement:

PR procedures consist of response requirements that progressively increase across a session until responding ceases. The last response requirement completed, termed "break point", provides a quantitative measure of the reinforcing effectiveness of a stimulus. For these studies, a session will be initiated by a colored box appearing in the middle of the screen. Touching the box once will result in food pellet delivery, and the response requirement will progressively increase following each reinforcer by an incremental value beginning at 1 and doubling after 8 response requirements are completed successfully. Thus, the response requirement sequences will consist of: Increment= 1, response requirements= 1, 2, 3, 4, 5, 6, 7,

8; Increment= 2, response requirements= 10, 12, 14, 16, 18, 20, 22, 24; Increment= 4, response requirements= 28, 32, 36, 40, 44, 48, 52, 56; and so on. Sessions will be a maximum of 10 min, and will be terminated if 3 min elapses without a response. Performance will be measured as the break point and number of food pellets delivered per session. This procedure provides a measure of the ability of the animal to respond, as well as an assessment of "motivation" to perform.

2e.iii. Scheduling of sessions: Initially, animals will be trained on either the PR task or the ID/ED task. These tasks are generally less demanding than the other CANTAB tasks, which should facilitate habituation of the animals to the touch-screen system. Once stability is achieved for these tasks, each of the CANTAB tasks will be presented once per day. An example of a typical sequence is: PR, SOSS, DMNS, PAL, ID/ED. Test sessions will last at maximum 3 hrs per day, and the compounds will be administered i.v. 5 min prior to the session. Two intervening sessions will be scheduled between tests to assure that no residual effects of the compound influence subsequent test results. In order to assure stable behavior, each task will be trained to designated performance criteria for each task. Training for cognitive tasks can take anywhere from 1 month to over 1 year, depending on the complexity of the task. Even when behavior is stable, "remedial" training is required in order to maintain stable performance. Thus, these studies are long-term, with monkeys trained or tested during the 5-day work week essentially during the entire year.

All CANTAB tasks will use the restraint chair and chamber system, in which the monkey is removed from the home cage each day at approximately the same time and placed into the experimental chamber. After the session, the monkey is returned immediately to the home cage. The non-CANTAB tasks take place in the home cage, using equipment designed to fit onto the cage. For the ORD tasks, sessions will occur 5 days per week, at approximately the same time, and generally last ~ 1 hour. For the NOR task, during habituation the objects are placed on the cage for times as brief as 5 min/day to long periods up to 24 hr/day. Behavioral measures are taken immediately after objects are placed on the cage.

2.f. Activity monitoring:

Activity will be monitored noninvasively using Actiwatches. Actiwatches are small, lightweight accelerometers that quantify and record movements. They will be placed in a protective case attached to commercially available nonhuman primate collars or to the pocket/extension in the back of the monkey's jacket either under light ketamine anesthesia (10 – 20 mg/kg, i.m.) or while awake and seated in a restraint chair (see Appendix G for details). Neither the case nor the monitor is in direct contact with the animal. This method has been used extensively to quantify activity in nonhuman primates over extended periods of time with no adverse effects. The collar, Actiwatch case or Actiwatch device do not impede the animal's movement in any manner. Both collar and case are typically well-tolerated by monkeys. Animals will remain in their home cages and no other changes will be made.

For Actiwatch maintenance, the animal will be lightly sedated (ketamine: 10 – 20 mg/kg, i.m.) or seated in restraint chairs and the Actiwatch removed in order to download data, change batteries, and reprogram. Actiwatches can record from 45

to 180 days, depending on data collection settings, without needing to be removed. We expect that no subject will be anesthetized more frequently than once per month to remove or place a monitor, although they may be removed earlier for health (e.g. skin irritation beneath the collar), equipment (e.g. loosening of screws holding the Actiwatch case to the collar), or experimental (e.g., cessation of data recording) reasons. Some subjects will wear those monitors continuously.

2.g. Tail-dip testing:

This procedure is widely used as a test of analgesia in rhesus monkeys, and includes placing the lower portion of an animal's tail into heated water. Analgesia is operationalized in this procedure as an increase in the latency to withdraw the tail from the heated water. In the absence of analgesics, the latency is shorter, and this latency is lengthened by analgesics (e.g., prescription opioids) in a dose-dependent manner. For our tests, the latency to remove the tail completely from the water, or a maximum of 20-s, will be measured. We use a 20-s cutoff to limit exposure of the tail to the heated water as a conservative protective measure. Notably, the tail-dip is an escapable situation. Subjects can easily withdrawal their tails from the water at any time. For this test, subjects will be seated in primate restraint chairs and transported to an experimental chamber for testing.

2.g.i. Training: We will shave approximately 10-15 cm of hair off the bottom of each subject's tail, either while they are restrained in the chair or while they are anesthetized with ketamine. Prior to testing, subjects will be habituated to the procedure by exposing the tail to room-temperature water in the same thermos that will be used for tests. It should be noted that this is not a training procedure, but rather an exposure to the test situation to minimize the effects of novelty on latency to withdraw. Put plainly, we do not want to the "surprise" of water on the tail to determine the latency to withdraw the tail from the water. This process will be repeated until the subject exhibits stable withdrawal latency behavior. Following this, testing will begin. A warm water bath will maintain water at a predetermined temperature (range non-noxious: 38°C to noxious 55°C; Maguire et al., 2013; Manning et al., 2001). The temperature of the water bath will be tested continuously with a thermometer to ensure temperature accuracy. For the test, water from the water bath will be placed in a thermos, and the subject's tail will be placed into the thermos. We will record the latency to withdrawal the tail (i.e., when the tail is completely removed from the thermos), or a maximum of 20 s, using a handheld timer/stopwatch (Banks et al., 2010; Maguire et al., 2013). If the maximum of 20 s is reached, the experimenter will immediately pull the subject's tail out of the water. At the frequencies, temperatures, and maximum duration of each exposure (i.e., 20 s) that will be used, no tissue damage is expected to occur.

2.g.ii. Testing: Tests will occur in cycles, with up to three tests (three instances where the animal's tail is dipped into water) per cycle. Within a cycle, no more than one test will be conducted with higher temperatures (i.e., 53-55°C). If more than one test is conducted within a cycle, one may occur with an intermediate temperature (i.e., 48-52°C), and one test may be conducted with a non-noxious temperature (e.g., 38-42°C). Cycles will last a minimum of 15 min, and a maximum of 6 cycles will be conducted on test days. Test days (i.e., days where animals are exposed to noxious

temperatures) will be conducted no more than three days per week, and will not be conducted on consecutive days (i.e., at least one day without testing between test sessions). Training days, with non-noxious temperatures (38-42°C), may be conducted between test sessions.

Drug or vehicle injections (IM, SC, or IV) may occur prior to a cycle. Depending on the particular test, drug administration may occur prior to one of the cycles or prior to many of the cycles with a maximum of six drug administrations (one per cycle). This dosing procedure is standard for this assay and allows a complete dose effect curve to be generated in a single test day (Banks et al., 2010; Maguire et al., 2013; Manning et al., 2001), and thus, reduces the overall duration of the study.

2.h. Multiple sleep latency test (MSLT):

Monkeys will be implanted with EEG/EMG/EOG telemetry implants and chronic i.v. catheters, attached or not to subcutaneous vascular access ports, as described in detail in Appendix C. Animals without subcutaneous vascular access ports are habituated to wearing a custom-designed nylon jacket (see Appendix G for details).

2.h.i. Training: Monkeys are first trained to sit in a restraint chair and are to wear a custom-designed nylon jacket, with no jacket being necessary if using the port system (see Appendix G for details). Animals are also habituated to the chamber in which MSLT trials will be performed. Prior to testing, all monkeys are submitted to implantable telemetry surgery, as described in detail in Appendix C.

2.h.ii. Testing: For the duration of the MSLT session (total of 5 sessions distributed throughout the day; session lengths combined not to exceed 3.5 hours per day), animals are positioned in a primate chair (Crist Instruments, Inc.; Primate Products style-custom manufactured) and placed in a dark, ventilated, sound-attenuating experimental chamber. Prior to MSLT sessions, the telemetry implant will be turned on by directly (direct contact with the skin) touching the area in the animal's back where the telemetry implant is located with a magnet while the animal is sitting in the chair. A "power on" device is used to ensure the implant was turned on properly. Once turned on, the implanted device communicates with a computer located inide the testing room through a receiver placed outside of the operant chamber. EEG/EMG/EOG readings are recorded in the computer through the use of a specialized software.

Experiments involve placing the animals in the chamber at 2-hour intervals: 8 AM, 10 AM, 12 PM (noon), 2 PM, and 4 PM. Sleep onset will be determined as the time to the first 30-second epoch scorable as sleep (based on EEG/EOG/EMG parameters scored real time by an experienced scorer), then the lights will remain extinguished for an additional 20 minutes of observation/scoring. If no sleep is observed, then sleep latency will be designated as 20 minutes. Thus, for each trial, animals will be in the chair/chamber for no longer than 40 min. Between naps, animals will be returned to their home-cages. At the end of each data collection period, devices will be turned off remotely through the software installed in the computer located outside of the room. These studies allow us to determine the animal's sleepiness under baseline conditions or in response to different drug treatments, with drugs being administered intramuscularly, subcutaneously or intravenously (catheterized monkeys) either before the first (8 AM) MSLT session of

the day or on the night before a day of MSLT sessions. MSLT sessions will be conducted no more frequently than 3 days per week.

2.i. Daytime and nighttime EEG/EMG/EOG recording:
Monkeys will be implanted with EEG/EMG/EOG telemetry implants and chronic i.v. catheters, attached or not to subcutaneous vascular access ports, as described in detail in Appendix C. Animals without subcutaneous vascular access ports are habituated to wearing a custom-designed nylon jacket attached to a tether and swivel (see Appendix G for details).

For this study, animals will remain in their home-cages, and a series of drugs will be administered intramuscularly, subcutaneously or intravenously (catheterized monkey) either during the daytime or prior to bedtime and EEG/EMG/EOG recording will be conducted. Prior to administration of drugs, the telemetry implant will be turned on by directly (direct contact with the skin) or indirectly (through the side of the cage) touching the area in the animal's back where the telemetry implant is located with a magnet. A "power on" device is used to ensure the implant was turned on properly. Once turned on, the implanted device communicates with a computer located outside of the room through a receiver placed in the center of the room. EEG/EMG/EOG readings are recorded in the computer through the use of a specialized software.

At the end of the data collection period, devices will be turned off remotely through the software installed in the computer located outside of the room.

3. If an unexpected problem or event occurs in the performance of the above described behavioral training/testing procedure(s) that directly impacts the live animal, what steps will be taken to ensure appropriate treatment is provided?

Although the experimental conditions of our studies should not result in the development of physical dependence, in the unlikely event that mild withdrawal-like indications develop, diazepam (1 – 3 mg/kg, b.i.d., i.m., or to effect) will be administered immediately to alleviate the physical symptoms. A CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

4. Will animal be observed/attended throughout the duration of the trial/test?
   ☒No ☐Yes
   If No, provide rationale.

In virtually all of our studies, the presence of an observer would change the behavior and add an uncontrolled variable to the experiment. Additionally, self-administration sessions can last for up to 9.5 hours (home-cage self-administration). Given the limited resource of technician time, it is unreasonably wasteful to staff a constant observer. Importantly, animals are observed pre- and post-session by trained observers.

5. Describe any unique post-trial animal husbandry that may be required (e.g., dry/warm environment for animals in the Morris Water Maze, soft padding for animals on the Rod Test, etc.).

No unique or special post-trial animal husbandry is necessary.

6. List personnel involved with the actual training and indicate his/her level of knowledge as it relates to the training/testing used in the lab.

[redacted] - 25+ years' experience
[redacted] - 25+ years' experience
[redacted] - 10+ years' experience
[redacted] - 5+ years' experience
[redacted] - 7+ years' experience
[redacted] - 13+ years' experience
[redacted] - 5+ years' experience
[redacted] - 4+ years' experience
[redacted] - 2+ years' experience
[redacted] - 2+ years' experience
[redacted] - 2+ years' experience

7. Where will the test(s) be conducted?

All testing will occur in the CCR – rooms [redacted]

8. Will the Animal Behavior Core (ABC) be used for this testing?
   - ☒ No
   - ☐ Yes – Use of the ABC requires review and approval by the Core Director.

X_____
ABC Director (Paste digital copy of signature)

Attach copies of ABC SOPs that will be used for this study.

Dear [redacted]:

Thank you for providing the information requested for your three-year full submission protocol, *Behavioral Pharmacology Studies in monkeys: Sedatives, analgesics, and stimulants,* considered at the August meeting of the Institutional Animal Care and Use Committee (IACUC). On **September 18, 2019** the IACUC approved the revised protocol. This protocol is assigned protocol number **1389B.** This protocol will remain valid until September 18, 2022 provided Annual Renewals are submitted as required.

Approval of your animal protocol does not imply that the protocol is congruent with any grant since the IACUC does not review a corresponding grant or grant application during the review of an Animal Activity Protocol. Congruency verification between a protocol and a grant is conducted when a grant submission or transfer is routed from the Office of Sponsored Programs to the Office of Animal Welfare for assurance verification. At that time there is a side-by-side comparison of an application/proposal and the IACUC protocol. Should any inconsistencies exist, you will be contacted. Congruency verification is a requirement of the NIH Public Health Service that must be met in order to maintain UMMC's Institutional Animal Welfare Assurance.

The Animal Activity Protocol form is recognized as a binding agreement between the Principal Investigator and the institution (via the IACUC). This document is designed to address the unique information relative to the animal study, as required by the USDA's Animal Welfare Act and the NIH/ OLAW's Public Health Service Policy on Humane Care and Use of Laboratory Animals. The IACUC recognizes that over the life of an Animal Activity Protocol the experimental studies with animals may "drift" or take seemingly minor departures from their original, documented plan. These deviations from the original IACUC-approved protocol are considered issues of noncompliance if they have not been previously covered by an amendment to the original protocol. All Principal Investigators and their staff are requested to review and become familiar with the IACUC policy that governs how instances of noncompliance are addressed by the IACUC. This policy, *Management of Suspected Protocol Noncompliance*, is available for review on the IACUC web site: https://intranet.umc.edu/sites/Research/Animal_Welfare/Documents/management%20of%20suspected%20protocol%20noncompliance.pdf

Reference should be made to the protocol number when animal orders are placed or when inquiries are made about this protocol. Committee approval of your protocol does not assure timely availability of animal housing space. Animal housing availability is coordinated through the Center for Comparative Research, ext. 4-1385.

A copy of the approved protocol is included with this communication. Please save a copy of the electronic file with your protocol documents for future reference. The IACUC encourages you to make a copy of the protocol available electronically and/or in paper format to your laboratory staff for reference.

Sincerely,

[redacted]

[redacted]
Chair-Institutional Animal Care and Use Committee

[redacted]

Institutional Animal Care and Use Committee
2500 North State Street • Jackson, Mississippi 39216
Phone: 601.815.5000 • Fax: 601.815.5010 • umc.edu