The University of Mississippi Medical Center

Animal Activity Protocol

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

Contents

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To be completed by IACUC

Protocol Number: **0486G**	Date: **02/13/2020**	Classification: **D**

1. Principal Investigator

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

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]	Assistant Professor	[redacted]	[redacted]	☐
[redacted]	Lab Manager	[redacted]	[redacted]	☒
[redacted]	Professor	[redacted]	[redacted]	
[redacted]	Researcher II	[redacted]	[redacted]	
[redacted]	Graduate student	[redacted]	[redacted]	
[redacted]	Postdoc	[redacted]	[redacted]	
[redacted]	Researcher III	[redacted]	[redacted]	
[redacted]				Added (10/7/20)
[redacted]				Added 9/16/21
[redacted]				Added 11/09/2021
[redacted]	Graduate Student			Added 2/22/22
[redacted]				Added 2/22/22
[redacted]	Researcher II	[redacted]	[redacted]	Added 5/2/22
[redacted]	Associate Professor		[redacted]	X
[redacted]	Research Volunteer		[redacted]	Added 6/3/22
[redacted]	MD/PhD student		Added 6/24/22	Depart 7/1/22
[redacted]	Researcher III		[redacted]	Added 7/25/22

(Insert additional lines as needed)

3. Project Title:

Behavioral pharmacology studies in nonhuman primates

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	Deterrents for prescription opioid abuse		
PI	[redacted]		
Funding Agency	NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R01DA039167

Covered Dates	9/15/15-5/31/20

Title	Unpredictable availability as a determinant of drug-related outcomes		
PI	[redacted]		
Funding Agency	NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R01DA045011
Covered Dates	4/1/18-3/31/23		

Title	Benzodiazepine choice and polydrug use		
PI	[redacted]		
Funding Agency	NIDA		
Status	☐ Submitted ☒Funded	Grant Number	R01DA054177
Covered Dates	4/1/22-3/31/27		

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

☐ Department – List Department: Click here to enter text.

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

7. Dates of Study

Anticipated start date of study: 2/1/2020

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 currently on campus 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
Rhesus monkey	**Macaca mulatta**	**M/F**	**Primate vendor or transferred from another UMMC IACUC protocol**	**48 + 8**	**30**	**18 + 8**	**40-45**

(Insert additional lines as needed)
originally approved for 48; 2/13/20

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.).

N/A

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 due to the presence of zoonotic viruses, and all protocols and procedures are maintained at Biosafety Level 2 (BSL-2). All disposable supplies that come in contact with monkeys are disposed of as biohazardous waste in Medical Waste (red bag) provided and serviced by Environmental Services (Ext. [redacted]). Surgical instruments are cleaned and autoclaved after each use. Personnel will be advised of special hazards and required to read instructions in CDC Guidelines on BSL-2 practices and procedures and required to follow them. All personnel will be required to read the biosafety manual, sign the UMC Statement of Informed Consent form, and demonstrate to [redacted] that they have understood its contents. [redacted] and [redacted] will ensure that protective clothing appropriate to BSL-2 hazards will be worn by all personnel at all times when performing all aspects of this protocol that involve close contact with either a living monkey or monkey tissue. This will include an eye shield, protective long sleeve clothing, mask and gloves. Should a person experience a monkey bite or scratch, or should monkey waste or potentially contaminated equipment come in contact with an open wound or a mucous membrane, that person will follow the established UMMC guidelines for such exposure as per CCR protocols posted in the animal facility.

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**:

Nonstandard Feeding: Each monkey in this protocol is fed by our personnel to ensure stable body weights. Drug and food intake can change with body weight. In some experiments monkeys lever press for food pellets, m&m candies, reese's pieces, skittles, Jell-O, or a sweet solution (saccharin, sucrose, Tang) in addition to drugs. In those monkeys, it is necessary to restrict food access to motivate lever pressing. A veterinarian or veterinary technician will determine a body condition score for each monkey with a goal to maintain a weight that renders a body condition score of approximately 2.5 to 3. Food amounts are adjusted in collaboration with veterinary staff to maintain animals at that target weight, and animals' body scores will be assessed approximately quarterly (i.e., when sedated for TB tests) and in some cases, more frequently. Animals are maintained at the target weight by control of food intake (food earned during a session plus supplemental feeding with monkey chow) and monitoring hydration status and overall animal health in consultation with veterinary staff. Target weights are often adjusted, for example, if an animal has a body condition score below 2.5 at the target weight, the target weight will be increased, usually by approximately 0.5 kg, until a new target weight is determined. In more extreme cases (i.e., when body scores fall below 2), the target weight and food rations will be increased more aggressively and may be supplemented by extra food items such as extra fruit, protein and fat dense foods, boost or ensure, etc. New target weights and food restriction are closely monitored and will be conducted in consultation with veterinary staff. Amount of food that is fed and consumed is documented daily on each individual animal's data sheet and on CCR forms to ensure that food consumption is easily monitored by veterinary staff. Monkeys with i.v. catheters are weighed approximately every two weeks and monkeys without i.v. catheters are weighed approximately every month. However, if concern regarding a monkey's weight is expressed by veterinary staff or by a PI, monkeys may be weight more frequently. Body weights are recorded on individual animal data sheets and charts are maintained on laboratory bulletin boards so that veterinary staff can easily monitor current body weights and trends over time for the entire colony.

Nonstandard caging: All animals are housed in standard, carter2 or similar, cages that allows visual, olfactory, auditory, and in some cases, tactile contact with other animals. Caging is considered nonstandard because animals are singly housed. Justification is below in 12b.

Nonstandard light cycle: An altered light cycle will be in place for rooms containing animals in self-administration studies. In these rooms, a 14/10 light/dark cycle will be in place. Some self-administration sessions continue into the early evening, and we have observed that animals' responding is slowed or stops in the dark. A 14-h light period will generally allow sessions to be completed during the light period.

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.**

Monkeys receiving regular intravenous injections of test compounds (either experimenter-administered or self-administered by the monkey) require surgically-implanted instrumentation (chronic indwelling intravenous catheters and jackets with tethers to protect the externalized infusion line) that is not compatible with social housing conditions (e.g., two co-housed subjects with separate tethers could easily become entangled or one could remove surgically-implanted instrumentation from cage mate). Moreover, the presence of other monkeys would interfere with drug taking during test sessions (our critical dependent variable).

Monkeys without catheters may receive test compounds that are not delivered intravenously (e.g., oral, i.m., s.c.) or may work for food pellets during experimental sessions. For these animals, a tether may not be necessary (and is a refinement to our procedure). However, these conditions will be compared to conditions in which an i.v. catheter/jacket/tether system is present. They will remain singly housed because 1) the presence of other monkeys could alter experimental outcomes in these control conditions and 2) may interfere with behavioral observations or with food taking during test sessions (our critical dependent variables)

To optimize social enrichment, cages will be grouped together in colony rooms in order to allow visual, auditory, and olfactory contact with other monkeys. Tactile contact between adjacent cages is also possible depending on the compatibility of individual monkeys. Animals that are not in a study and that are not instrumented with chronic indwelling catheters may be socially housed if there is sufficient time between studies for the stepwise integration required for the social pairing of rhesus monkeys (approximately three months or longer).

13. Housing

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

☒ No

☐ Yes Where?

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

N/A

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.

The main objective of our research is to understand factors that contribute to substance-use disorder, with the overall goal of improving treatment. To do this, we use food and drug self-administration to determine the abuse potential of new drugs and drug combinations or to determine the effectiveness of certain drugs and alternative nondrug reinforcers (such as food or liquid) at reducing or enhancing the rewarding effects of established drugs of abuse such as cocaine or heroin. We use tail withdrawal as an assay to determine the potential effectiveness of drug and drug combinations at treating pain. Finally, we use behavioral observation to determine the extent to which these drugs and drug combinations produce observable side effects such as sedation. Collectively, our experiments are aimed at reducing drug abuse, maintaining the clinical effectiveness (i.e., treatments for pain) of prescription drugs that are often abused, and ensuring that our drug/drug combinations have reduced side effects or an acceptable side-effect profile.

15. Rationale

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

Substance-use disorder is a biobehavioral disorder that is determined by a complex interaction between an organism, a drug, and an environment. The work described in this protocol cannot be conducted in humans because of the addictive and experimental nature of the drugs under study. Many of the compounds have not been evaluated in human subjects. The research also cannot be conducted solely using tissue samples or biological material 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 drug taking by organisms at this time.

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

Rhesus monkeys have played a major role in drug abuse research for several decades, first in studies of physiological dependence, later in studies of drug self-administration, drug discrimination, and tail withdrawal, and recently in behavioral observation. This species is ideally suited for preclinical research on drug abuse, and for many questions in addiction research are considered the “gold standard” species, particularly by federal regulatory agencies such as the FDA and DEA. Since monkeys are phylogenetically closer to humans than are rodents, for example, the results of studies with monkeys may be more directly generalized to humans. Their higher level of intelligence and complex interactions with the environment make it possible to model more closely the interactions between drug taking and

the environment that drives addiction in humans. Moreover, the longevity of rhesus monkeys allows for long-term studies of the effects of chronic drug exposure as well as studies of behavioral history that contribute to drug abuse. Finally, rhesus monkeys have a menstrual cycle that essentially parallels that observed in humans, and this fits well with new initiatives to evaluate sex differences in addiction.

16. Brief Outline

Provide a general descript. ion of the animal procedures included in the experimental design. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.

- **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.

Note : For the four procedures described below, details of experimental design are described in Appendix L.

Self-administration

Food and drug self-administration studies are ongoing in our lab throughout the year, and they do not adhere to a specific timeline because multiple drugs (and doses within a drug) can be tested in a single subject for the duration of a catheter's patency life (often 2+ years per catheter) and over the course of the life of the organism (10+ years). This within-subject approach for our experimental design allows for a significant ***reduction*** in animal numbers, which would otherwise be exponentially higher if we were to test every dose of every drug in separate groups of monkeys**.**

Tail withdrawal assay

Tail withdrawal from warm water is used to measure the analgesic effects of test drugs. Drugs that produce pain-decreasing effects will result in a longer latency to remove the tail from submersion in a container of heated water. Similar to self-administration, tail-withdrawal tests do not adhere to specific timelines or rigid experimental plans because multiple drugs and doses within each drug may be tested in a single subject. Moreover, subjects that are tested in self-administration to screen for the abuse liability of analgesic drugs (e.g., opioids) may also be tested in the tail withdrawal assay to determine if novel opioids that exhibit lower abuse liability still reduce nociception.

Observation studies

A key component of testing novel drugs for abuse liability and analgesic effect is determining if they produce other unintended effects (i.e., side effects such as sedation or pruritis). We

have established a quantitative scoring system that allows us to screen for a number of observable effects in monkeys that are indicative of drug side effects in humans. As with the study designs above, these tests do not adhere to a specific timeline or rigid experimental plan for the reasons described above. Typically, drugs are administered (either experimenter administered or self-administered) and trained observers measure species-typical ("normal") behavior, as well as characteristic drug-related behavior, such as sedation. Experimenters occasionally interact with the monkeys to assess for sedation-like behaviors. This includes calling the animal's name, tapping the cage, and recording the animal's response to the assessment.

Drug administration: In all experiments, drugs may be self-administered via the IV or oral routes and may be experimenter administered via IV, IM, SC, or PO routes. For IV delivery, the drug is administered through a surgically implanted catheter. For IM delivery, an injection is given in the hamstring or quadricep and when multiple injections are given within the same day, alternating locations are used. For SC delivery, the injection is given under the skin by gently pulling up loose skin, usually on the animal's leg or back, to make a tent. The needle is inserted at the base of the tent. For oral delivery, the drug is mixed with Jell-O or a sweetened solution (e.g., juice, tang). Animals readily consume the solutions from a syringe or sipper. With Jell-O, the experimenter can simply hand the gelatin to the animal as a treat.

Collection of biological samples Alongside each behavioral assay, animals may be trained to receive vaginal swabs. We will train animals for collection of 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 (see Appendix L). Once training is complete, we will take vaginal swabs up to but no more than once per day. Monitoring the menstrual cycle is necessary to conduct on a daily basis initially, and may be collected less frequently if fluctuation in the menstrual cycle is minimal from cycle to cycle. Daily collections will allow us to precisely determine menses onset/offset which will allow us to predict menstrual cycle phase
In addition, during cage change outs and under sedation, at least once, a microbiome sample of the gastrointestinal tract, mouth and nasal cavity will occur by swabbing the rectum along with stool collection, and swabbing the mouth and nasal cavity

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).

Because our experimental design uses a subject as its own control (within-subjects design), we are able to minimize the number of monkeys in an experiment (usually 4-6 monkeys). Although this repeated-measures design prolongs experiments, it has both scientific and practical benefits. When a subject is used as its own control, as opposed to using a different subject as a control, an experimental manipulation need only have a very small effect to be detectable. This is a clear scientific benefit given the biological and behavioral variability inherent to a genetically diverse population of primates. Within-subjects designs also have the benefit of significantly decreasing the number of monkeys needed for any given experiment thereby limiting the laboratory population of this valuable resource. This rationale is supported by power analyses indicating a sample size of N=4 as sufficient for identifying differences in

our dependent measures (α=0.05, power=0.80; G*Power version 3.0.10), including the power to detect between-subject sex differences.
The PI was recently awarded a new grant to study benzodiazepine choice in a polydrug scenario (e.g., in subjects with a stimulant, opioid, benzodiazepine, or no drug experience/history). We request to add 8 naïve rhesus monkeys to our protocol. We already have most of the subjects for our food, benzodiazepine, and cocaine experienced groups in our current colony. We do not have any animals with appropriate histories for the opioid group. We plan to use 6 of the newly purchased animals for the opioid group and to add one male to our cocaine group and one female to our food group. The groups will not be compared with each other per se, rather the experiments will be completed as separate studies for each group with n=5/6 per drug or food experience group.

18. Location & transportation

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

Room Number	Procedures performed
[redacted], or other housing room in the CCR	Self-administration, warm-water tail withdrawal, behavioral observations, and vaginal swabs.
Dedicated surgical suites in CCR	All surgical procedures

(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 CCR (e.g. IVIS, surgery room).**

Monkeys will be transferred from housing areas in the [redacted] (Basement) to the nearby surgical suites. A cart will be used to transport the anesthetized monkey through the hall.

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?

When all available veins have been used, animals may be moved to other studies/protocols or euthanized.

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 CCR, ext. [redacted], for recommendations on the assessment criteria.

Upon recommendation of the veterinary staff, for example, if an animal is deemed terminally ill. Importantly, CCR veterinary staff makes daily (M-F) observations of each nonhuman primate and partners very closely with our lab to gauge overall health status.

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 CCR.)

In the event that euthanasia is necessary, overdose with a commercially available euthanasia solution at packaged labeled doses or at doses to effect will be given intravenously, and pneumothorax with tissue harvest for necropsy by a veterinarian will be used as the secondary method. These procedures are consistent with the recommendations of the Panel on Euthanasia of the AVMA Guidelines on Euthanasia (2013). In all cases, euthanasia is conducted by CCR staff.

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:

Warm-water tail withdrawal test	

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 (Click here to enter text.)

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	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **replacement**	☒	☐	☐	☐
AWIC	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **replacement**	☒	☐	☐	☐
Pubmed	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **reduction**	☐	☒	☐	☐
AWIC	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **reduction**	☐	☒	☐	☐
Pubmed	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **refinement**	☐	☐	☒	☐
AWIC	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **refinement**	☐	☐	☒	☐

Pubmed	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **opioids**	☐	☐	☐	☒
AWIC	Dec. 2019	All years to present	Warm-water tail withdrawal	Warm-water tail withdrawal AND monkey AND **opioids**	☐	☐	☐	☒

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 search did not reveal any approaches incorporating reduction, replacement, or refinement into the study of the analgesic effects of drugs. A number of papers were identified using the warm-water tail withdrawal in monkeys to study the analgesic effects of novel opioids. However, we are studying recently-developed molecules within the opioid class that have yet to be studied in nonhuman primates. Thus, the work we will conduct under this protocol will not be duplicative.

Reductions in animal number:

We reduce the number of subjects by using a within-subject design and by using subjects in multiple experiments.

Refinements to methods to reduce distress:

Personnel handling animals provide daily enrichment and are trained to conduct manipulations in a manner that minimizes distress. Our surgical procedures are conducted using aseptic techniques and appropriate analgesics are administered to minimize pain. Our research personnel use positive reinforcement techniques to gradually train animals in behavioral procedures to allow animals to fully adapt to each step before progressing to the subsequent phases.

Animal **Replacement**:

Because our behavioral assays are by default a whole-organism measure, there are no alternatives to the use of animals.

Training and Qualifications

- PI

Name► [redacted]
Animal research experience ► > 20 years experience with rodents and > 10 years experience with nonhuman primates

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
Self-administration	> 10 years
Tail withdrawal	~5 years
Behavioral Observation	~5 years
Surgery	> 10 years

Name► [redacted]
Animal research experience ► >10 years with rodents and >7 years with nonhuman primates

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	>7 years
Tail withdrawal	~5 years
Behavioral Observation	~5 years
Surgery	>7 years
Collection biological samples	> 2 years

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

Name► [redacted]
Animal research experience ► > 5 years

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	> 3 years
Tail withdrawal	> 3 years
Behavioral Observation	> 3 years
Surgery	> 3 years
Collection biological samples	> 3 years

Name► [redacted]
Animal research experience ► ~ 6 months with nonhuman primates

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	~ 6 months
Tail withdrawal	~ 6 months
Behavioral Observation	~ 6 months
Surgery	~ 6 months
Collection biological samples	~ 6 months

Name► [redacted]
Animal research experience ► > 5 years with rodents, > 2 years with nonhuman primates

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	> 2 years
Tail withdrawal	> 2 years
Behavioral Observation	> 2 years
Surgery	> 2 years
Collection biological samples	> 2 years

Name► [redacted]
Animal research experience ► > 5 years with rodents, > 5 years with nonhuman primates

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	> 2 years
Tail withdrawal	> 2 years
Behavioral Observation	> 2 years
Surgery	> 2 years
Collection biological samples	> 2 years

Name► [redacted]
Animal research experience ► > 2 years with rodents, > 2 years with nonhuman primates

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Self-administration	> 2 years

Tail withdrawal	> 2 years
Behavioral Observation	> 2 years
Surgery	> 2 years
Collection biological samples	> 2 years

Name► [redacted]
Animal research experience ► at least 2 years of experience working with rodents. No work with nonhuman primates, though. They will be trained for this work.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Drug self-administration	TBT
Behavioral observation	TBT
Catheterization surgery	TBT

Name► [redacted]
Animal research experience ► at least 2 years of experience working with rodents. No work with nonhuman primates, though. They will be trained for this work.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Drug self-administration	TBT
Behavioral observation	TBT
Catheterization surgery	TBT

Name► [redacted]
Animal research experience ► None

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol
Food and Drug self-administration	To be trained
Behavioral observation	To be trained

Name► [redacted]
Animal research experience ► [redacted] has 3 weeks of experience working with 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
Shadowing only. No procedures.	

Name► [redacted]
Animal research experience ► None

Qualifications to perform specific procedures

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

Food and drug self-administration	To be trained
Behavioral observation	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"

[redacted], [redacted], or [redacted], my lab manager who has over 5 years' experience working with nonhuman primates in my lab, will train new hires in the use of drug self-administration and behavioral observation.

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.

[redacted]

X_____

Signature of Principal Investigator (Paste digital copy of signature)

Approval by the Attending Veterinarian:

X [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.

The CCR staff and our personnel provide an active plan of environmental enrichment; e.g., social interaction in the form of visual, auditory and grooming interactions, cage complexities (tunnels, barrels), fruit/vegetables, foraging, manipulative devices/toys, radio, and TV. Enrichment will be documented in writing by the person actually giving it to the monkey. The form for documentation is on the room door.

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 housing is requested (see parent protocol, section 12b, for justification).

Exemption from other forms of enrichment:

Appendix B Time-Pregnant/Breeding Programs

Complete Appendix B for all proposals planning on establishing a breeding colony or for those studies utilizing time-pregnant animals. Studies incorporating breeding programs or offspring from time-pregnant animals will be required to report annual production (number of offspring used) at the time of IACUC protocol annual renewal.

1. Description

a. Provide a specific description of the type of breeding program to be utilized (monogamous pair, "trio" breeding: 2 females and 1 male, "harem" breeding: up to 4 females and 1 male, etc.).

b. The *Guide for the Care and Use of Laboratory Animals* sets minimum space requirements for breeding animals. *See chart below.

   If you wish to request a deviation from the minimum requirement provide justification based on performance standards (e.g., health, reproduction, growth, behavior, activity, and use of space) and special needs determined by the characteristics of the animal strain or species (e.g., obese, hyperactive) and experimental use (e.g., animals in long-term studies may require greater and more complex space).

c. For all mating schemes other than pair breeding, pregnant females must be separated prior to birth of the litter unless an exemption is justified. If using trio or harem breeding, please describe how/when dams will be separated to ensure that overcrowding does not occur.

d. All litters must be separated at 21 days of age unless an exemption is justified. Please describe specific plans for weaning.

2. Personnel Responsible

Identify personnel responsible for the breeding program, including weaning and documentation of program.

3. Records

Please describe the record-keeping system that will be used and how breeding, health and maintenance of the colony is recorded.

4. Adults

a. How many adults will be utilized in this breeding program over the 3 year period?

b. How many breeding pairs/groups will be utilized at one time (may be explained with a range)?

c. How many breeding cycles will be utilized or what is the maximum length of breeding (e.g., 3 breeding cycles or 1 year)?

5. Final Disposition

What is the final disposition of these adults at the conclusion of their breeding program?

6. Offspring

How many offspring are anticipated from each breeding or time-pregnancy?

7. Final Disposition

What is the final disposition of any offspring not utilized in the experimental program (e.g., euthanasia, replacement of retired breeders, transferred to another protocol)?

8. Genotype

Describe the sample collection method used for genotyping animals, including age at time of genotyping. Include tissue sampled in Appendix D.

9. Phenotype

Will any offspring have any known or anticipated clinical health concerns (immunocompromised, severe diabetes, ataxia, prone to dermatitis, etc. see also #9.c.)?

*Guide for the Care and Use of Laboratory Animals: Eighth Edition
http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf

TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups*

Animals	Weight, g	Floor Area/Animal,[a] in.^{2} (cm^2)	Height,[b] in. (cm)	Comments
Mice in groups[c]	<10	6 (38.7)	5 (12.7)	Larger animals may require more space to meet the performance standards.
	Up to 15	8 (51.6)	5 (12.7)	
	Up to 25	12 (77.4)	5 (12.7)	
	>25	≥15 (≥96.7)	5 (12.7)	
Female + litter		51 (330) (recommended space for the housing group)	5 (12.7)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d]
Rats in groups[c]	<100	17 (109.6)	7 (17.8)	Larger animals may require more space to meet the performance standards.
	Up to 200	23 (148.35)	7 (17.8)	
	Up to 300	29 (187.05)	7 (17.8)	
	Up to 400	40 (258.0)	7 (17.8)	
	Up to 500	60 (387.0)	7 (17.8)	
	>500	≥70 (≥451.5)	7 (17.8)	
Female + litter		124 (800) (recommended space for the housing group)	7 (17.8)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d]

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
The surgical site and back are thoroughly scrubbed with an aseptic surgical scrub (e.g., betadine), wiped away with 70% ethanol, and scrubbed with prep solution.
Surgical approach
Intravenous catheterization: Monkeys have 8 veins that we may catheterize (one vein at a time): left and right brachial, external jugular, internal jugular, and femoral veins. Subjects are given atropine and ketamine, followed 10-20 min later by inhaled isoflurane. When anesthesia is adequate, a 1-1.5 inch incision is made above vein tissue. The tissue is blunt dissected and the vein is isolated. Suture is passed under the vein, and the piece distal to the heart is tied. A small cut is made in the vein and a catheter is inserted into the vein. A piece of suture is tied around the vein wall, over the catheter to help keep the catheter in place. In addition, the catheter is tied to adjacent muscle tissue with suture that is glued to the catheter or tied around cuffs that have been glued to the catheter. The distal end is passed subcutaneously with a probe to exit between the scapulae. The catheter is pulled to an optimal length. Sensorcaine (0.25%) or another pharmaceutical grade topical anesthetic is applied to the incision site, and the incision is closed. Antibiotics and analgesics are given as indicated in the table below or as directed by a veterinarian. **Catheter removal:** When a catheter is no longer functional (e.g., blockage or out of the vein) or if an animal becomes ill (e.g., catheter tract infection), the implanted catheter will be removed. To begin, the monkey is given ketamine as an initial anesthetic. Before proceeding to inhaled isoflurane, the catheter is gently pulled from the outside of the monkey. If the catheter slides out easily, it is removed without an incision. If the catheter does not pull out, the monkey is prepped as described for **intravenous catheterization.** An incision is made above the site of the insertion of the catheter. The sutures that tie the catheter into the muscle are exposed by blunt dissection and are cut. The catheter is then pulled from the vein, any remaining suture material is removed, and the incision is sutured. Antibiotics and analgesics are given as indicated in the table below. **Catheter replacement:** If a catheter becomes blocked, and there is no infection, we will try to replace the catheter. The animal is prepped, anesthetized, and given the same care as described above for an **intravenous catheterization.** An incision is made above the site whether the catheter enters the vein, the sutures that tie the catheter into the muscle are exposed by blunt dissection and are cut. The vein and old catheter are also isolated via blunt dissection. The old catheter is pulled out, and an attempt is made to slide the new catheter into the vein. If successful, the new catheter is anchored and guided to the exit site as described for **intravenous catheterization.** The incision is closed and antibiotics and analgesics are given as indicated in the

table below. This procedure often allows us to continue to use a vein, ultimately reducing animal use.

Catheter reprobe: Occasionally, the skin opens above a catheter tract or a catheter is internalized. In these and similar cases, in consultation with veterinary staff, the catheter is extended if internalized and in all cases, moved to a new subcutaneous tract. For this procedure, the monkey is anesthetized and incision site prepped as described for **intravenous catheterization.** An incision is made along the catheter tract (proximal to the skin opening in cases where a catheter has exteriorized), the catheter is isolated and exteriorized using blunt dissection. The distal end of the catheter is cut off, and new catheter is attached to the original, joined with a short piece of sterile, stainless steel tubing. The catheter ends are secured to the tubing by tying short pieces of catheter material around the connection. The distal end is then passed subcutaneously to a new exit sit, along a new catheter tract. The incision is closed and antibiotics and analgesics are given as indicated in the table below. This procedure often allows us to continue to use a vein, ultimately reducing animal use.

Wound closure method, materials, and removal plan

Sensorcaine (0.25%) or another pharmaceutical grade, topical anesthetic is applied to the wound, and the wound is closed with subcutaneous, continuous vicryl suture. All sutures are buried/subcuticular to prevent “picking”. No removal will be required since vicryl is absorbable.

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

	Agent	Dose	Route	Frequency/Duration	Pharmaceutical Grade
Pre-anesthetic	Ketamine	5-20 mg/kg	i.m.	once	☒Yes ☐No
	Atropine	0.04 mg/kg	i.m.	once	☒Yes ☐No
	Veterinary staff may use alternative drugs for animals that are not easily sedated with ketamine				
Pre-operative analgesics	Carprofen	2-4 mg/kg	s.c., i.m., or p.o.	Once pre-surgery then repeated once daily as needed, usually 3 days	☒Yes ☐No
Post-operative analgesics	Buprenorphine SR	0.05 mg/kg	s.c.	May be given with veterinary recommendation	☒Yes ☐No
Anesthetics	Isoflurane	1-5%	Inhaled	Continuous	☒Yes ☐No
Fluid/blood replacement	Saline	0.9%	i.v.	As needed	☒Yes ☐No
Antibiotics	Keflex or other as recommended by veterinary staff	20-25 mg/kg	s.c., i.m., or p.o.	Once pre-surgery then, when and as recommended by veterinary staff, once or twice daily (depending on the antibiotic) for one week or as	☒Yes ☐No

				recommended	

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 staff or other certified veterinary technician

b. Describe experience and training with anesthesia.

CCR staff and certified veterinary technicians are trained via their education and certification to administer anesthesia and monitor animals during surgical procedures

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

Anesthesia is administered and monitored by a certified veterinary technician or a veterinarian. Criteria used are heart rate, respiratory rate, muscle tone, oxygen saturation, and body temperature.

4. Aseptic Technique

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

The surgeon and any assistants will scrub their hands about ½ way down their arms with a betadine pad/scrubber. A sterile towel is used to dry hands and arms. The surgeon and assistants then put on a sterile gown and gloves. All methods (i.e., hand washing, putting on gown and gloves) are conducted according to methods taught by CCR during training seminars.

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

Disposable items are either ordered in a sterile form or are autoclaved for each surgery. Instruments are soaked in Enzycare for the time period indicated on the packaging post surgery. They are then wrapped and autoclaved after each use

5. Location of Procedures

Where will the surgical procedures be conducted?

CCR surgical suite in [redacted], or on [redacted] when the basement is not available

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 personnel and CCR veterinary staff will conduct post-operative monitoring according to the CCR post-op form

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

Animals are observed continuously until extubation, then approximately every 15 minutes until conscious. They are also observed approximately hourly for several hours after surgery, then 2-3 times/day for a total of 72 hours. Observations are recorded and initialed on the post-op observation sheet posted on the room door. Carprofen is given for analgesia as needed, usually once daily for 3 days post surgery. Laboratory personnel or CCR staff may administer antibiotics as directed by CCR staff depending on the procedure conducted and veterinary recommendation.

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

Animals are returned to their home environement immediately after being removed from isoflurane (usually as soon as the jacket is placed on the animal, and the system is connected to catheter).

7. Emergency Contacts

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

[redacted] cell: [redacted] Office: [redacted] or on campus extension: [redacted] [redacted] cell: [redacted] Office: [redacted] or on campus extension: [redacted]

Appendix D	**Collection of Biological Samples from the Live Animal**

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

Vaginal discharge; Microbiome via rectum/mouth/nose swabs, collection of feces

2. Indicate the method and site of collection.

Vaginal discharge will be collected for evaluation of the presence/absence of menstruation or cells consistent with different phases of the menstrual cycle. Awake sampling will be required because of the frequency of sample collection (up to once/day). The monkey will be trained to present her hindquarters. We will also use the method of "successive approximations" to train animals to present their hindquarters, then allowing the trainer to touch the skin near the vaginal opening with a cotton swab, and so on).

Samples will be collected from each animal under sedation during standard veterinary care and/or during cage change outs following the methods developed and used in the Human Microbiome Project. Not only does this allow for collection of minimally perturbed microbiomes, but also ensures consistency across collection sites and dates as well as providing the ability for direct comparison with best practice human studies. Full details on sample collection can be found in publications from the Human Microbiome Project and as adapted for non-human primates in our previous work. All samples will be collected using a sterile flocked ESwab™ or eNAT™ (Copan Diagnostics) or a BactiSwab™ (ThermoFisher Scientific). After collection, the swab is then inserted into a collection tube (Fig1). The tube is labeled and transferred to [redacted] for DNA isolation. The microbiome of the gastrointestinal tract is sampled at the rectum and through stool collection; the oral microbiome through swabbing the tongue dorsum, hard palate (roof of mouth), and buccal mucosa (cheek) (Fig2); and the nasal microbiome by swabbing the mucosal surfaces of the anterior nares (Fig3).

Figure 1

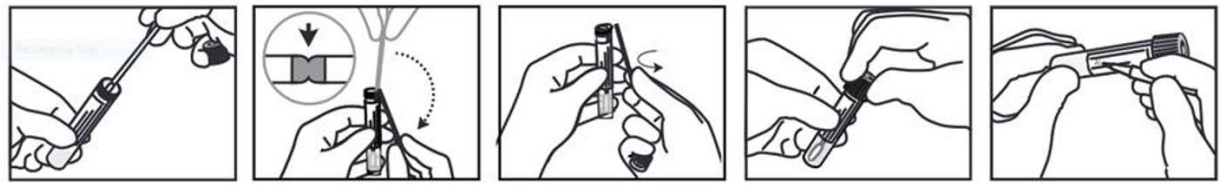


Figure 2

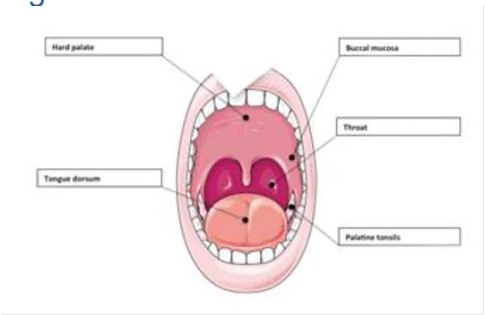
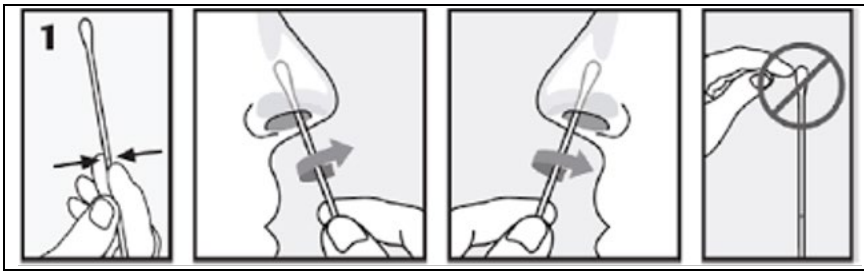


Figure 3



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

A single swab with a cotton tipped applicator for the vaginal swab; single swab with cotton tip applicator for each type of sample collected from rectum, mouth, and nose swab

4. Indicate the frequency of collection.

Vaginal swabs may be collected on a daily basis to track menses onset/offset which can be used to correlate with different phases of the menstrual cycle. Vaginal swabs may be continuous with female subjects in the approved protocol. Even for subjects not currently on study, we will collect vaginal swabs so that we can detect regularities or irregularities in an animal's menstrual cycle and whether menstrual cycles change across time or during certain experimental conditions.

Rectum, mouth, and nose swabs will be collected once per animal. In some cases, if DNA isolation does not work, a second sample may be collected.

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.)

For vaginal swab, no restraint is necessary, animals are trained to present for swab collection using positive reinforcement

For rectum, mouth, and nose swabs the animal will be sedated during the procedure as part of routine care.

If Yes, list agents used for anesthesia and anaglesia:

Agent	Dose	Route	Frequency/Duration	Pharmaceutical Grade
Ketamine	5-20 mg/kg	i.m. or i.v.	once	☒Yes ☐No
				☐Yes ☐No

For non-pharmaceutical-grade compounds:

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

N/A

d. 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 E Antibody Formation /Hybridoma & Ascites

1. Indicate what antigen will be used: Click here to enter text.

2. Indicate what vehicle/adjuvant will be used: Click here to enter text.

   a. Initial immunization: Click here to enter text.
   b. Subsequent immunizations: Click here to enter text.
   c. Anticipated complications/side effects: Click here to enter text.

3. Indicate sites for immunization: Click here to enter text.
4. Describe skin or animal preparation prior to injection: Click here to enter text.
5. Indicate route of administration: Click here to enter text.
6. What is the total and per site injection volume? Click here to enter text.
7. What is the frequency/duration of immunization (e.g., 1 injection every 2 weeks for 3 injections)? Click here to enter text.

ASCITES PRODUCTION

Fluid accumulation associated with ascites/hybridomas should not become greater than 10% of body weight. Animals should be euthanized if they become moribund.

8. Indicate the maximum volume of ascites fluid to be collected per sampling (ml/mouse) and the method of collection (skin prep, gauge needed, gravity vs. suction, etc.)

9. Indicate the number of fluid collections and anticipated frequency of collection.

10. Describe procedures used to care for and monitor the health of animals with ascites and the point of euthanasia.

Consult: http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf
http://oacu.od.nih.gov/ARAC/documents/Ascites.pdf

Appendix F Administration of Drugs/Test Compounds

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:

Agent	Dose	Volume	Vehicle	Route	Frequency	NDC o CAS#	Hazard?	Pharmaceutical Grade
Stimulants	0-1.0 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility and purpose of experiment	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week[3]		Yes	☐Yes ☒No
Depressants	0-10 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility/ purpose of experiment	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week		Yes	☐Yes ☒No
Benzo-diazepine agonists and antagonists	0-30 mg/kg/ injection	≤3.0 ml	Saline/sterile water when possible, though usually one or more of approved	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered**		Yes	☐Yes ☒No

			vehicles (below) is necessary for solubility reasons		**(IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week			
Mu and Kappa Opioid agonists and antagonists	0-10.0 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility and purpose of experiment	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week		Yes	☐Yes ☒No
Procaine	0-30 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility and purpose of experiment	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week	51-05-8	Yes	☐Yes ☒No
Niacin	0-10 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility and purpose of experiment	IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week	59-67-6	Yes	☐Yes ☒No
Histamine	0-0.1 mg/kg/ injection	≤3.0 ml	Typically saline, though any approved vehicle (below) may be used depending on solubility and purpose of experiment	IV, IM, SC	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week	51-45-6	Yes	☐Yes ☒No
Methohexital	0.1-3.3 mg/kg/ injection	≤3.0 ml	Saline or sterile water	IV	As needed to test catheter patency, generally not more than once a day	4202 3-105-01	Yes	☒Yes ☐No
Ketamine	5-20 mg/kg/ injection	≤3.0 ml	Saline	IV, IM	As needed to test catheter patency or anesthetize an animal, generally not more than once a day	1867-66-9	No	☒Yes ☐No
Vehicles								

09% Sterile Saline	-	≤3.0 ml		IV, IM, SC	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week		no	☒Yes ☐No
Sterile water		≤3.0 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week		No	☐Yes ☒No
Ethanol	≤ 20% of a solution	≤ 3 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week	62991-1663-01	No	☐Yes ☒No
Propylene glycol	Up to 100% of a solution	≤ 3 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week	57-55-6	No	☐Yes ☒No
Tween 80 / cremaphor	≤ 10% of a solution	≤ 3 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week	9005-65-6	No	☐Yes ☒No

Benzyl Alcohol	≤ 20% of a solution	≤ 3 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week	100-51-6	yes	☐Yes ☒No
Beta-cyclodextrin	≤ 45% of a solution	≤ 3 ml		IV, IM, SC, PO	**Self-administered (IV,PO):** Determined by animal, ~30 injections/day **Experimenter administered (IV,IM,SC,PO):** ≤6 injections/day, ≤4 times/week OR 1 administration/day, up to 7 times/week	7585-39-9	No	☐Yes ☒No
Jello-O or sweet liquid solution (i.e., juice)	Up to 100% of a solution	≤ 20 ml		PO	**Self-administered**: Determined by animal, ~30 deliveries/day **Experimenter administered**: ≤6 deliveries/day, ≤4 times/week OR 1 administration/day, up to 7 times/week		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.).

For all compounds listed in the protocol, no adverse events are expected at the doses, frequencies, and routes of administration. However, potential side effects are listed for particular drug classes below. 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.

Stimulants and procaine: These drugs can suppress appetite, and at large doses, can induce seizures. 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.) 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.

Depressants and benzodiazepine agonists: These drugs can 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 or to effect, i.m.)**.** These drugs can also result in physical dependence with associated withdrawal syndrome. For the studies described in this protocol, physical dependence would be a confounding factor and is not planned. All experimental conditions are planned to limit the likelihood for dependence. That is, we limit the number of consecutive sessions that can be conducted in a single week, and we maximize the total number of injections delivered. 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, reduced appetite). In the unlikely event that mild withdrawal-like indications develop, diazepam (1-3 mg/kg or to effect, i.m., i.v., or p.o.) will be administered to alleviate the physical symptom, 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.

Opioid agonists: These drugs can cause constipation, ataxia, sedation, and at high doses, respiratory depression. Naltrexone (0.1-1 mg/kg or to effect, i.v. or i.m.) will be administered for suspected opioid overdose and in some cases, following test sessions if sedation is observed. Naltrexone will be stored in solution in an unlocked locker and/or in the room in which the experiment is being conducted so it is readily available and can be delivered as quickly as possible. All research personnel are trained to deliver naltrexone. Opioid agonists can also 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 that is not a planned experimental endpoint. All experimental conditions are planned to limit the likelihood for dependence. That is, we limit the total number of injections delivered, and for animals receiving an opioid on a daily basis, we use the smallest dose possible. For all studies, monkeys will be observed by trained staff immediately after the experimental sessions for sedation and/or withdrawal signs (diarrhea, laying down in the cage). Withdrawal symptoms may be treated with diazepam as described above for depressant and benzodiazepine withdrawal.

Histamine: This drug can cause appetite suppression which can, in some instances, be alleviated by appropriate adjustment of food type and allotment in consultation with CCR veterinarians. Monkey weights and food intake are monitored carefully, and if appetite suppression lasts longer than one week, we will lower the dose or suspend testing with histamine. It is important to note that this side effect is quite rare at the dosages used in the protocol.

Ketamine and Methohexital: these drugs will be administered at doses and concentrations, and by routes of administration within proven safe parameters in

monkeys and are not expected to produce an adverse side effects that would require medical attention or endanger the health of the animal

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?

1

<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 tail withdrawal. This requires that, whenever possible, we administer pure forms of the compound without additional solvents that may have intrinsic effects (e.g., glycine), and b) acquire a form of the drug that can be dissolved into sterile solution for intravenous delivery. 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.

   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).

All final solutions are passed through a 0.2 μm Millipore filter into a sterile container. The pH of all compounds are tested prior to administration to ensure that the solution is within the 5.5-8.0 range, with 5.5 being the typical pH of sterile physiological saline before compounds are mixed. Doses administered are behaviorally active but not administered within a toxic range. In addition to micro-filtration at the time of drug preparation, all compounds administered via an IV route pass through an additional 0.2 μm Millipore filter prior to entering the animal. This filter is attached to each animal's syringe and is changed approximately every 2 weeks.

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.

Food and self-administration and observation experiments are long-term studies. Restraint is necessary to maintain a functional i.v. administration preparation. The data from monkeys that do not have i.v. catheters are directly compared to data from monkeys with i.v. catheters. Therefore, the behavioral situation, including restraint, may be comparable. For studies that do not require an i.v. preparation, the jacket/tether restraint system may be removed once we have confirmed that similar results are obtained with and without the jacket/tether.

Tail withdrawal procedures are conducted in a restraint chair for two reasons. First, the chair allows the experimenter to safely acces the monkey's tail. Second, when administering compounds via an IV route, it allows the experimenter to safely access the catheter to administer experimental drugs/compounds in a way that is safe for the experimenter and the monkey (this way the catheter is less likely to be reached by the monkey, and is therefore less likely to be pulled by the monkey and can be manipulated using sterile technique.). For this assay, monkeys typically are in the chair for no more than 1-2 hours before being returned to the homecage.

2. Describe the restraint device.

Monkeys are fitted with stainless-steel restraint harnesses or mesh jackets with tethers (for food/drug self-administration, observation) that attach to the side of a standard cage. In rare cases, a harness may be attached over a jacket to prevent the subject from pulling out of the jacket, or a jacket may be placed over a harness to prevent manual access to the externalized portion of a catheter. In all cases, there is no postural restriction and they have full access to all areas of the cubicle or cage. This is a relatively non-restrictive form of restraint.

Monkeys in the tail withdrawal assay may be fitted with mesh jackets with a pocket to store the catheter portion that exists the back, but no tether. Restraint chairs are custom made from 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.

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

Food and drug self-admininstration and Behavioral Observations: A harness or jacket is placed on the monkey in a standard cage, and at least one week is allowed for adaptation to the harness or jacket before initial training. Using this process, monkeys are usually adapted to handling within two weeks.

Animals adapt well to these procedures, and laboratory personnel take care to use shaping and positive reinforcement and expose animals to new things gradually. Problems in transition rarely occur, but when they do they are evidenced by a transient disruption in eating, self-directed biting, aggressive behavior such as lunging or swiping at personnel or the hook. These cases are resolved with longer adjustment phases (e.g., to the harness, cubicle, jacket training) and exposures at a slower pace than normal.

Tail withdrawal: These animals are fitted with collars and may be fit with mesh jackets and allowed to acclimate for at least 1 week as described above for self-administration and behavioral observation. 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?

Food and drug self-administration and Behavioral Observations: Continuous while in training or in an experiment. Between studies, during a transient period of removal from a study, or if results from conditions without jacket/tether/i.v. system yield similar results as jacket/tethered conditions (as described above), the tether and jacket may be removed.

Tail withdrawal: Monkeys will remain in restraint chairs for the duration of test sessions. Test sessions typically last 1-2 hours and will not to exceed 3 hours per day. Post-test session, animals will be returned to their standard caging.

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

Food and drug self-admininstration and Behavioral Observations: Continuous while in training or in an experiment.

Tail withdrawal: daily, up to 5 times/week (mon-fri)

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

Food and drug self-admininstration and Behavioral Observations: Animals are monitored on a daily basis by laboratory staff and CCR staff. Monday through Friday, excluding holidays, animals are observed a minimum of three times/day. On days that sessions are not conducted, animals are monitored a minimum of twice/day (once by laboratory personnel and once by CCR staff).

Tail withdrawal: This task is not automated, therefore, the animals are monitored continuously while in restraint chairs.

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

Harnesses may cause redness or pressure sores in spots with skin contact. If redness or sores occur, vet staff will be consulted. Typically, loosening the harness will relieve the sore. Sometimes padding is required. Occasionally, the harness must be removed.

There is also the possibility of skin lesions develop under collars or mesh jackets (e.g., around shoulders and underarms or belly area). We minimize this possibility by adjusting the jacket at the neck and belly to be as loose as possible, yet still protect the catheter. Monkeys will be checked for lesions by laboratory staff every 1-2 weeks until laboratory and CCR staff are confident that the collar and/or jacket fit is appropriate. After initial fitting, we will check for lesions every time an animal is sedated. Harnesses may be used as an alternative to jackets if problems with lesions cannot be resolved (and vice versa).

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 survival surgical events in a single animal.

There are a number of major veins that can be catheterized for self-administration. For this

reason, we can *reduce* the number of animals needed for self-administration studies by using multiple veins in serial fashion. That is, when a catheter and vein lose patency, we can place a catheter into a different vein and continue studies within an animal rather than order another animal.

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

Typically, a catheter will last a year or more in jugular (external or internal) and femoral veins, and about half that time for brachial veins. Thus, inter-surgical intervals are typically set according to these patency timelines. However, sometimes a catheter can become blocked at a subcutaneous point before vein entry and can be repaired by splicing a new span of catheter material to the proximal side of the clot, which requires incising the skin near the point of the clot and making the repair subcutaneously. Events such as these typically happen well into a catheter's "lifespan", so the duration of inter-surgical intervals for these repairs would still typically be on the order of 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 some experiments, lever pressing is maintained by food pellets, m&m's, reese's pieces, skittles, or flavored liquid. To motivate animals to lever press for food, body weight will be decreased to maintain a body condition score (BCS) of approximately a 2.5-3. An animal's weight will be maintained by food earned during the session and by supplemental feeding of monkey chow.

Level of food deprivation can also influence drug self-administration behavior. Animals in experiments that only involve drug reinforcers will have their food scheduled and weights monitored to ensure that feeding or becoming overweight does not interfere with behavior during the session.

Finally, rhesus monkeys allowed access to food ad lib most often become obese. Food will be scheduled and/or restricted to prevent a monkey from becoming obese with the goal of maintaining animals at a body condition score of 3.

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

hydration.

METHOD		FREQENCY OF CHECKS
Body weight	☒	Approximately every 2 weeks at cage change for animals with an i.v. preparation and approximately every month for animals without an i.v. preparation. If an animal displays reduced appetite, becomes ill, or veterinary staff or PIs express concerns regarding a low body weight, they may be weighed more frequently
Urine output	☒	daily
Fecal output	☒	daily
BUN	☐	
Hct	☐	
Food intake	☒	daily
Other	☒	Veterinary staff obtain body condition scores during regularly scheduled tb tests, and may obtain body scores more frequently

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?

We try to order subjects in their young-adult years. When we are able to do this, we get a baseline weight and body condition score (BCS) for approximately 4-6 weeks (e.g., while they are in quarantine). In addition to weights, CCR veterinary staff examines each monkey's body condition during quarantine and at least once approximately every 3 months thereafter. Feeding is adjusted with the aim of maintaining a BCS that is approximately 2.5-3.0. To mitigate bias, staff assigning BCSs are encouraged to do so before seeing a current weight, as this information may lead to under- or over-estimation of BCS.

When younger monkeys are used, we reduce body weight to a body condition score of approximately 2.5-3 and then allow them to grow gradually (about 1 kg/year until adult) to about 9-12 kg. Free-feeding male rhesus monkeys can range in weight from about 7-15 kg and free-feeding female rhesus monkeys can range in weight from about 5-12 kg. Adjusting food amounts according to BCS allows us to accommodate weight gain related to natural growth while ensuring the organism's body condition is relatively consistent for the duration of a study.

What is the maximum restriction for any animal?

Our restriction is a body score of approximately 2.5-3. However, if an animal is not lever pressing for food pellets or liquids, we may reduce the animal's food ration, usually by 1 biscuit (15g) at a time, until lever pressing for food pellets is maintained. If an animal's body condition falls below a score of 2.5 we will increase that animal's food ration and treat them in consultation with veterinary staff.

An animal may be NPO'd for 12 or 24 hours before starting lever-press training, however, they will be maintained as close to their target weight as possible thereafter.

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?

Body weight will be allowed to increase by approximately 1 kg/year until adulthood and body condition will be monitored approximately once every 3 months.

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

Animals will be fed their ration once daily. Food left over will be counted and recorded on each animal's data sheet and on data sheets posted in the basement for veterinary staff. Water intake will not be restricted, however, the water spouts in each animal's home environment will be checked daily to ensure that they are working properly.

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

Animals will be on the regulation protocol continuously. In the event that an animal is not in an experiment, it will still be necessary to regulate food intake to prevent the animal from over eating and becoming obese.

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

Animals will have unrestricted water in the homecage at all times.

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

Laboratory personnel listed in this protocol will be responsible for administering and documenting the regulation.

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 J Animal Pain and/or Distress

The management of post-procedural pain or distress is typically addressed with the use of

appropriate pharmacologic and non-pharmacologic methods (see Appendix C). Appendix J should be completed if there are any procedures that are proposed that may cause more than momentary, slight pain or distress during which the appropriate sedatives, analgesics, or anesthetics will be **withheld** or in **which chronic pain or distress is induced**. Proposals which incorporate animal manipulations or procedures which may create more than momentary pain and distress (noxious injections, tumor growth, sequelae to compound administration, etc.) should also be addressed. For additional information consult the IACUC's policy on Animal Pain and/or Distress.

1. **Justify** the scientific need to withhold appropriate drugs or induce the pain/distress.

2. What is the duration of time that an animal may experience this pain/distress?

3. Describe non-pharmaceutical means to alleviate pain/distress (soft bedding, social housing, supplemental heat, etc.).

4. Describe situations where an animal may be removed prematurely from a study.

5. Describe those procedures whereby animals are likely to experience more than momentary pain or distress as a result of manipulations or procedures (noxious injections, tumor growth, sequelae to compound administration, etc.).

6. Will any anesthetics, analgesics, or tranquilizing drugs be used to reduce this pain or distress?

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.

Over the past three years we have extended our knowledge of substance-use disorder by extending previous work investigating the determinants of drug choice and in establishing foundational work for developing safer opioids for the treatment of pain.

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

Publications

[redacted] Choice between variable and fixed cocaine alternatives in male rhesus monkeys. *Psychopharmacology*, 234:2653-2364, 2017.

[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] Kappa opioid agonists reduce oxycodone self-administration in male rhesus monkeys. *Under Review.*

[redacted] Quantification of observable behaviors induced by kappa opioid agonists in male rhesus monkeys. *Under Review.*

Abstracts

[redacted] Comparison of the punishing effects of nalfurafine and salvinorin A on cocaine and oxycodone self-administration in rhesus monkeys. *Oral communication at the Kappa Therapeutics Conference*, Philadelphia, PA, April, 2017.

[redacted] Behavioral profile of mu and kappa-opioid agonists in male rhesus monkeys. *Poster presentation at the College on Problems of Drug Dependence,* San Diego, CA, June, 2018.

[redacted] Oxycodone self-administration on a progressive-ratio schedule is reduced by contingent administration of the atypical kappa-opioid agonist, nalfurafine, in rhesus monkeys. Oral communication at the College on Problems of Drug Dependence, San Diego, CA, June, 2018.

[redacted] Variability as a determinant of food and cocaine choice in rhesus monkeys. *Poster presented at the Association for Behavior Analysis International's Substance Use and Addiction Conference*, Washington, D.C., November 2018

[redacted] (May 2019) Delay discounting of food and co-caine in male rhesus monkeys. Paper presented at the 45th Annual Convention for the Association for Be-havior Analysis International, Chicago, Illinois.

[redacted] Quantification of observable behaviors induced by kappa agonists in rhesus monkeys: Effects of signaling bias. *Oral communication at the College on Problems of Drug Dependence*, San Antonio, TX, June 2019

[redacted] (March 2018) Uncertainty as a major factor underlying substance-use disorder. Department of Psychiatry Grand Rounds, University of Mississippi Medical Center, Jackson, Mississippi.

[redacted] (May 2019) Uncertainty as a major factor underlying substance-use disorder. Presentation at the Society for the Quantitative Analysis of Behavior annual meeting, Chicago, Illinois.

[redacted] (May 2019) Variability as a determinant of food and cocaine choice in rhesus monkeys. Presentation at the thematic session on Choice at the 45th Annual Convention for the Association for Behavior Analysis International, Chicago, Illinois.

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

   ☐ Yes ☐ **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.).

We occasionally have monkeys that show a progressively decreasing appetite. In these cases, the health of the monkeys is monitored closely by veterinarians. In this

past period, we had one case in which the decision was made to euthanize the monkey. Irregularities in organ form (enlarged liver with discoloration) were identified in necropsy that may have been contributing factors to the declining health in this subject.

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?

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:

Personnel occasionally receive scratches from monkeys or equipment with which monkeys have had contact. These cases are documented in student-employee health and are treated using an SOP for primate exposure.

3. What treatment measures were taken:

We follow an SOP for treating primate exposures that is approved by CCR and Employee Health.

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?

Food and drug self-administration, tail withdrawal, behavioral observation, and vaginal swabs

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.).

When monkeys arrive at UMMC, they are held in quarantine for at least 6 weeks by the CCR. During quarantine the laboratory staff of [redacted] and [redacted] may interact with the monkeys, offering treats and acclimating monkeys to staff and equipment. During the last weeks of quarantine, staff may also sedate, weigh, and fit the monkeys with stainless-steel restraint harnesses or mesh jackets. Accepting the presence of the staff will be positively reinforced with hand-delivered treats. These interactions will be documented on the CCR form attached to the room door.

Food and drug-self administration: Following quarantine, monkeys may begin self-administration studies. Tests are conducted daily in the home cages (Carter2). Cages are equipped with modular cartridges that can be attached to the sides of the cages. In inner face of the cartridge contains two horizontally-adjacent levers, associated stimulus lights, and trough for the delivery of 1-g food pellets (BioServe). The interior of the cartridge contains instrumentation for interfacing with computer, up to two syringe pumps (Med Associates) for the delivery of solutions, and a carousel-style food dispenser (Med Associates) for the delivery of food pellets. Drug injections and/or food pellets are delivered contingently with responses on the levers to which they are assigned. Monkeys are trained to press a lever that results in the delivery of food, liquid, or an intravenous injection of a drug or drug combination (see Appendix F). Taping a raisin to the lever that delivers the reward is usually enough to establish the behavior of lever pressing. For experiments involving behavior that is maintained by food, body weights are maintained at a target weight that results in a body condition score of approximately 2.5-3. Weight is maintained by food delivered during experimental sessions and supplemental feeding of monkey chow. Lever presses for rewards (food or drug) serve as the test data, and test sessions occur 5-7 days/week depending on the experiment.

Tail withdrawal: For this test, subjects will be seated in primate restraint chairs located within the housing room. Training animals to be chaired is described in detail in appendix G. We will shave approximately 10-20 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. A warm water bath will maintain water at a predetermined temperature (range non-noxious: 38°C to noxious 55°C). 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 withdraw the tail (i.e., when the tail is

completely removed from the thermos), or a maximum of 20 s for temperatures up to 52.5°C or 15 seconds for 55°C, using a handheld timer/stopwatch. If the maximum time is reached, the experimenter will immediately pull the subject's tail out of the water. 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, up to two tests may occur with an intermediate temperature (i.e., 48-52°C) with a third test at a non-noxious temperature or one test at a noxious temperature may be conducted with two tests at 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 administrations (PO, 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/cycle). This dosing procedure is standard for this assay and allows a complete dose effect curve to be generated in a single test day and thus reduces the overall duration of the study.

Behavioral Observation: Behavioral measures, in a drug-free state or following administration of different compounds, alone or in combinations, will be determined using quantitative observational techniques. All observers will be unaware of the goals of the study as well as the compound(s) 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. Using this scoring system, the presence of a behavior is noted during each 15-sec interval and the number of 15-sec intervals during a 5-min session the behavior is observed is recorded. Thus, the maximum score for each behavior during one modified frequency sample is 20. Baseline behavioral profiles will be determined after monkeys habituate to the human observer (typically 1-2 weeks). Behavioral observations may occur in 5-min blocks during or following food and drug self-administrations or may occur as a stand-alone procedure. In the latter case, a range of 0-6, 5-min sessions may be conducted each day, and IV, IM, SC, or PO drug or vehicle administration may occur prior to each 5-min session. In cases when multiple sessions are conducted per day, this will allow for drug combinations to be evaluated or for cumulative dosing and determination of complete dose-response functions in a single day, and this may reduce the overall duration of the study. Importantly, these animals will not receive more drug administrations than typically occurs in our self-administration procedures. Drs. [redacted] and [redacted] have developed a scoring system for sedation and other species typical and drug-induced behaviors that we will use in our observation studies.

Vaginal swabs: Animals will be trained by laboratory personnel to undergo awake vaginal swab. Vaginal cells/discharge will be collected for evaluation of the presence/absence of menstruation or cells consistent with different phases of the menstrual cycle. The monkey will be trained to present her hindquarters. We will also use the method of "successive approximations" to train animals to present their hindquarters, then allowing the trainer to touch the skin near the vaginal opening with a cotton swab, and so on. Based on our experience with this approach, we anticipate training to take about 1 month. We will be able to examine cells from vaginal swabs under a microscope, or determine presence/absence of menstruation to determine menses onset/offset.

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?

Food and drug-self administration: In most experiments, we limit access to drugs, either by limiting availability to a few hours a day and/or programming a time-out between injections so that drug cannot accumulate to toxic levels. Monkeys do not usually experience any serious drug-induced side effects. The primary side effects of the preparation are septic in nature (e.g., catheter tract infections or septicemia). Any problems are treated through consultation with the CCR veterinary staff. Any stereotypical behavior that may develop will be addressed in consultation with CCR veterinary staff.

Tail withdrawal: 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, the animal will immediately be removed from the test apparatus and returned to the home cage. The temperatures, frequencies, and durations tested are in accordance with previously published research, and tissue damage is not expected. Veterinary staff will be alerted if redness or swelling consistent with burn damage is observed, and treatment will be provided according to veterinary recommendation.

Behavioral Observation: In observational sessions, we limit access to drugs to a maximum of six administrations/day or for observations during/following food and drug self-administration, drug access is limited as described above. Monkeys do not usually experience any serious drug-induced side effects. The primary side effects of the preparation are septic in nature (e.g., catheter tract infections or septicemia). Any problems are treated through consultation with the CCR veterinary staff. Any stereotypical behavior that may develop will be addressed in consultation with CCR veterinary staff.

Vaginal swabs: Because of the frequency vaginal swabs, we will interact closely with the CCR veterinarians in order to monitor the site of collection for vaginal swabs to ensure there is no evidence of inflammation caused by daily collections. Any signs of trauma will be considered indicators that the sampling

will be halted and re-initiated after veterinary approval.

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

Food and drug-self administration: Lever pressing occurs in an enclosed environment, and experimental events and data collection are controlled by a computer. Additionally, lever pressing can be disrupted by an observer or if individuals enter and exit the room. Additionally, drug self-administration sessions can last for several hours, especially when relatively long inter-infusion intervals are programmed, and the preparation is relatively safe. In the event of a power surge, or other unexpected electrical problem, each drug pump has a safety timer that does not allow a pump to run continuously. Timers are typically set at 10 s for syringe pumps.

For behavioral observation, tail withdrawal, vaginal smears, animals will be attended throughout the duration of the procedure

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.).

None

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.

Our research technicians will be primarily responsible for daily training and testing animals in consultation with [redacted] and [redacted]. All of our technicians have received several forms of training relevant to the behavioral procedures, and training is documented in standardized forms provided by UMMC's ORSP. Experience with specific tasks for each personnel member is described in the parent protocol under the section "Training and Qualifications".

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

Room Number	Procedures performed
[redacted], or other housing room in the CCR	Food and drug self-administration, tail withdrawal, behavioral observations, collection of biological samples (vaginal swabs)

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.

Appendix N Use of Expired Medical Materials or Devices

The use of expired medical materials and/or drugs may be allowed for non-survival procedures. The attending veterinarian and the IACUC are responsible for ensuring that proposed animal activities avoid or minimize discomfort, distress, and pain to the animal. These responsibilities cannot be met unless the veterinarian and the IACUC maintain control over the use of expired medical materials.

All anesthetics, for survival and acute procedures, analgesics, emergency drugs, and euthanasia agents must be in date.

All pharmaceuticals and medical materials (e.g. drugs, antibiotics, fluids, saline bags, disinfectant solutions, catheters, sutures, etc.) used in survival procedures must be in date.

For additional guidance see the IACUC Policy Statement *Use and Maintenance of Expired Medical Materials (Pharmaceuticals and Devices)*

1. List and describe expired medical materials and/or expired medical devices to be used and describe intended use of each item. *NOTE: All expired medical materials or devices must be clearly labeled, "Expired, for conditional use only".*

2. Please provide a justification for the use of the expired items.

3. Describe if sterility will be required, and if so, how proper sterility will be assured.

4. Identify the room and exact location where expired items will be stored. *NOTE: Items must be kept in a separate location (cabinet, shelf, box) and must be clearly labeled, "Expired, for conditional use only".*