cayuse Animal Oversight

UMMC

PI :	[redacted]
Protocol #	2022-1199
Status :	Approved (w/o Stipulation)
Approved :	07/11/2022
Expires :	07/11/2025
Title :	Non-human primate models of alcohol abuse: Behavioral pharmacology studies

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Protocol Introduction

Federal/Foundation funded?

☑

Internally funded?

☐

Private/Commercially funded?

☐

Other funding source?

☐

Will you be using tissues from animals not on this protocol?

Yes ○ No ◉

Will you be using human tissues?

Yes ○ No ◉

Will this study include antibody, hybridoma or ascites production?

Yes ○ No ◉

Will you be collaborating with an outside institution?

Yes ○ No ◉

Will any live animal research be conducted off campus?

Yes ○ No ◉

Will animals be housed outside central housing facilities for more than 12 hours?

Yes ○ No ◉

Will the study involve transporting animals to locations outside of the housing area, including transport within the CCR (e.g., IVIS, Surgery room)?

Yes ◉ No ○

Will human patient or clinical areas be used?

Yes ○ No ◉

Will you be using chemical, biological, or radiation hazards?

Yes ◉ No ○

Protocol Species Grid

To add a species, click the "ADD" button on the bottom of the grid, select the species from the picklist then place checkmarks
in each column as applicable. Save the species selection by clicking the Save button on the bottom of the grid, then save the
page by clicking the Save button below the grid.

Species	Breeding?	Procedures?	Prolonged Restraint?	Surgery?	Vet Drugs?	Exp. Agents?	Euthanize?	IBC Agents?
Rhesus Macaque	No	Yes	No	No	No	Yes	Yes	No

Protocol Overview

Enter title for this Protocol

Non-human primate models of alcohol abuse: Behavioral pharmacology studies

The response should be written in non-scientific language, as though explaining the study to a high school student. Generally, single-sentence explanations for these types of questions will suffice.

- In non-technical terminology, how would you explain to a non-scientist the long term or overall objectives of the proposed work?
- Why are the experiments proposed?

The objectives of our studies are to understand the role that specific brain proteins (GABA-A and opioid receptors) and genetic differences play in alcohol drinking and relapse. With this knowledge, we can identify better treatment strategies for individuals who misuse alcohol by itself or combined with other drugs of abuse.

The response should be written in non-scientific language, as though explaining the study to a high school student. Generally, single-sentence explanations for these types of questions will suffice.

- What is the potential relevance of experimental findings to human or animal health, advancement of knowledge, and/or the good of society?
- What knowledge do you hope to achieve?

Although many individuals have benefited from existing anti-alcohol medications, the effectiveness of these drug treatments are generally moderate, with some individuals unaffected by treatment. Further, there is evidence that individual differences, including genetic differences, can underlie differences in risk for developing an alcohol use problem and response to anti-alcohol medications. Finally,, there is growing evidence that opioids frequently are co-abused with alcohol and that this polydrug abuse can increase lethality of the individual drugs, as well as decrease the effectiveness of opioid maintenance therapy.

Through our studies, we hope to: 1) identify of novel molecular targets for the development of anti-alcohol medications, 2) define the role of specific genetic variation in risk to develop alcohol use problems, and 3) characterize the interaction between alcohol and opioids and determine whether particular individuals are more likely to abuse the drug combinatioin.

Provide a general overview of the animal studies proposed. This description should allow a non-scientist to understand the course of an animal from its entry into the experiment to the endpoint of the study. **Do not describe technical details in this summary. See the example below.**

Note that specific details about methods and procedures will be required in subsequent sections of the protocol form.

Example: Mice will be used to test different cancer treatments. Mice will be injected with cancer cells and tumor progression will be monitored three times per week for the duration of the study using imaging techniques that measure tumor growth. Beginning one week after cell implantation, mice will be treated with different therapeutic agents as often as every other day for six weeks. By the side of the end of the treatment period, mice will be euthanized by isoflurane overdose and tissues collected.

Abstract

Monkeys on this protocol will be trained in oral self-administration procedures. All monkeys also will be observed for species-typical and drug-induced effects. Once trained, the disposition of the subjects will depend on the specific study. Some monkeys will self-administer alcohol or sucrose solutions and be treated with different drugs targeting the GABA-A or opioid systems (each dose will be administered for 5 days or until behavior is stable). These studies will determine whether any test compound might have therapeutic effects against alcohol. Other monkeys will self-administer an opioid or a GABA-A compound solution, with and without alcohol. These studies will evaluate the co-abuse of alcohol with other drug classes. Other monkeys will enter protocols designed to assess relapse behavior (cue exposure, resurgence). These studies will identify conditions under which relapse occurs and will assess the effectiveness of drug treatments to block relapse. In some cases, monkeys will be "profiled" before or after studies. These profiles may include cognitive testing, assessment of blood alcohol levels, etc.

Protocol Federal/Foundation Funding List

Click "Add Funding Source" and complete funding information. If you are the PI, you may click "Add From My List of Funds" which will narrow the options to funding sources previously approved on your protocols. You may add more than one funding source.

Fund Source	Grant Title	Funded?	End Date	Grant #
National Institute On Alcohol Abuse And Alcoholism	GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol	Yes	07/31/2025	AA029023

If the funding source is not available in the drop list, contact the IACUC office to have it added.

Fund Source

National Institute On Alcohol Abuse And Alcoholism

Grant Title

GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol

Currently Funded?

☑

Grant Number

AA029023

PI on Grant (if different than PI on Protocol)

Proposed End Date

07/31/2025

Transportation of Animals

Transportation of animals outside the central facility must follow guidelines set by the IACUC.

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

Type of Animal Use

Identify all types of animal use for this protocol.
Your choices here and on subsequent pages will determine the correct USDA pain category.

Which of the following describe the type of animal use proposed in this application? (check all that apply)
Research Type (Research, Breeding, Sentinel)

Research

Other?

☐

Hazardous Use Info

Hazardous Agents

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 (https://documents.umc.edu/PolicyList.aspx).

Hazard Types *(check all that apply)*

For each hazardous material, a Hazard Use form must be completed and attached to the protocol. Link to Hazardous Use form (https://umc.edu/Research/Research-Offices/Office-of-Animal-Welfare/IACUC/form---hazardous-use.pdf).

Chemical Hazard

☑

Biological Hazard

☑

Radiation Hazard

☐

How many Hazard Use forms are attached?
The form is required for all hazardous agents. Please attach the completed form(s) after saving the page.

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Provide specific details of specialized animal husbandry, care, cleaning, or decontamination procedures, especially identifying responsible parties.

None of the experimental agents/chemical hazards require specialized animal husbandry, care, cleaning, or decontamination procedures. The same is true for the biological hazard (rhesus monkeys). All administration of experimental agents will be done by personnel associated with this protocol.

Rhesus Macaque

Animal Identification Method(s)

Animal Identification Method(s)

Rhesus monkeys generally arrive in the colony tattooed, either on their upper chest or inner thigh areas.

Strain Information

Choose a strain from the pick list. If the strain you want is not on the pick list, enter it in the other field.

Strain	Phenotype	Unique Phenotype	3 Year Total	Sex	Average Daily Census
rhesus monkey		N/A	36	Female/Male	26

Species Strain

rhesus monkey

Phenotype

If the strain will have a phenotype not described above, please describe the phenotype.
- Include clinical signs that will be monitored and frequency of monitoring.
- Describe how the animal will be treated to minimize pain and distress.
- Indicate when an animal will be removed from the study or euthanized (humane endpoints).

N/A

Total number of animals (3 year total).

36

Sex

Female/Male

Estimate of Average Daily Census.

26

Rationale for Species and/or Strain

Justify the choice of species and strain by stating why a species lower on the phylogenic scale or a different strain is not appropriate.

Provide a rationale for the choice of species and/or strain

Rhesus macaques are ideally suited for preclinical research on drug/alcohol addiction, and for many questions in addiction research are considered the "gold standard" species, particularly by federal regulatory agencies such as the FDA and DEA. This species has been used in behavioral pharmacology research for over 30 years and has provided valid and reliable models of multiple aspects of alcohol use disorders. For our GABA and opioid studies, data show that the distribution of specific receptors in the primate brain, but not the rodent brain, more closely mimics the distribution in humans. Additionally, for genetics research, rhesus monkeys are appealing as they exhibit naturally occurring polymorphisms in many genes that have functional consequences that parallel the consequences in humans. Finally, because of the extensive use of rhesus macaques in neuroscience research, there is a large body of scientific information which provides indispensable comparative information for proper interpretation of our research.

Species Source

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.

Will any animals be obtained from non-commercial sources?

Yes ◉ No ○

List the non-commercial animal source(s).

Monkeys initially were transferred from the New England Primate Research Center, Southborough, MA. Should animals need to be purchased for this protocol, they most likely would be sourced from other National Primate Research Centers. Subjects also may be transferred to this protocol from other UMMC investigators

Environmental Enrichment

The Center for Comparative Research provides an active plan of environmental enrichment that is species specific. Unless otherwise, specified, the CCR will provide all available forms of enrichment. Any exceptions to the standard enrichment plan should be described and justified in this section.

Are there any forms of enrichment/enhancement that should not be provided?

Yes ○ No ◉

Use Locations

Indicate all of the locations where surgeries, procedures and/or euthanasia will be performed.

Location/Building	Room	Procedures
Center for Comparative Research	[redacted]	Housing; oral self-administration/relapse procedures; behavioral observation; cognitive testing
Center for Comparative Research	[redacted]	Housing; oral self-administration/relapse procedures; behavioral observation; cognitive testing

Note: procedure details will be included in another section. Only provide procedure titles, not full details.

Location/Room

Center for Comparative Research [redacted]

Indicate the procedures that will take place at this location.

Housing; oral self-administration/relapse procedures; behavioral observation; cognitive testing

Note: procedure details will be included in another section. Only provide procedure titles, not full details.

Location/Room

Center for Comparative Research [redacted]

Indicate the procedures that will take place at this location.

Housing; oral self-administration/relapse procedures; behavioral observation; cognitive testing

Non-Surgical Procedures

Include procedures or biological sample collection from live animals. Do not include samples taken after euthanasia.

Procedure Name	Restraint Description
Activity Monitoring	Sedation (ketamine) is only needed when actiwatches need to be removed for downloading data.
Behavioral Observation	N/A
Blood Collection	
Cognitive testing	N/A
Human Intruder Test (HIT)	
Motor function test (mMAP)	N/A
Relapse Procedure	N/A
self Admistration	N/A

Procedure Name

Activity Monitoring

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

Activity will be monitored noninvasively using Actiwatches. Actiwatches are small, lightweight accelerometers that quantify and record movements. They will be placed in a protective case attached to commercially available nonhuman primate collars. The collar will be fitted on the animal under light ketamine anesthesia (10- 20 mg/kg, i.m.). This method has been used extensively to quantify activity in nonhuman primates over extended periods of time with no adverse effects. The collar and Actiwatch case do not impede the animal's movement in any manner.

Both collar and case are typically well-tolerated by monkeys. Animals will remain in their home cages and no other changes will be made.

For Actiwatch maintenance, the animal will be lightly sedated (ketamine: 10 ? 20 mg/kg, i.m.) and the Actiwatch removed in order to download data, change batteries, and reprogram. Actiwatches can record up to 180 days, depending on data collection settings, without needing to be removed.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

Sedation (ketamine) is only needed when actiwatches need to be removed for downloading data.

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Procedure Name

Behavioral Observation

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

Two-five times/week, trained observers will monitor the animals' behavior in order to establish quantitative behavioral profiles. No devices, training of the animals, food/fluid restriction or positive reinforcement are required. Behavior will be assessed using a modified frequency measure for each animal as described previously by [redacted] et al. (2002; Psychopharmacology 164: 151-159). A range of species typical behaviors, as well as drug-induced behaviors, will be recorded during 20 15-second intervals by trained observers (total of 5 min). Observers will be trained using a standard procedures manual and will complete at least 20 hrs of scoring prior to participating in the study. Percent agreement scores will be used to determine inter-observer reliability, with a criterion of = 90% required.

Using this scoring system, the presence of a behavior is noted during each 15-second interval and the number of 15-second intervals during the 5 minute session in which this behavior is observed are recorded. Thus, the maximum score for each behavior during one modified frequency sample is 20. This modified frequency scoring system controls for variability between animals by limiting the number of intervals in which a behavior can occur. Behaviors that will be scored are divided into two different types: Species-typical behavior and study-specific behaviors. Species-typical behaviors are based on observations of monkeys in naturalistic settings and will provide a quantitative analysis of the effects of genotype and/or alcohol on normal behavioral profiles. Study-specific behaviors will be characteristic alcohol- or other drug-induced effects, including different measures of sedation.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

N/A

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Procedure Name

Blood Collection

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☑

Describe the procedure, giving enough detail so that another individual could carry it out.

Monkeys will be lightly sedated with Ketaset (ketamine; 10-20 mg/kg, i.m.) for the collection of samples. 2-3 mls of blood will be collected from the femoral or saphenous vein.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ◉ No ○

Indicate the body fluid or material to be collected.

rhesus monkey blood

Indicate the method and site of collection.

Blood will be collected from the femoral or saphenous vein. Monkeys will be lightly sedated with Ketaset (ketamine) for the collection of samples.

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

2-3 mls

Indicate the frequency of collection.

In the normal course of an alcohol self-administration study, blood samples will be collected, at most, every two weeks.

Procedure Name

Cognitive testing

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

We will assess performance on three cognitive tasks: Object Retrieval with Detours (ORD), Object Discrimination Reversal (RL), and Novel Object Recognition (NOR). Testing is conducted by trained technicians and occurs in the animal's home cage with the devices mounted to the front of the cage. Descriptions of the devices are provided with the specifics of each test. No preliminary training, food or fluid restriction is necessary to induce the animals to perform the tasks. Rather, positive reinforcement is used. In the ORD and RL tasks, monkeys are rewarded with food treats (e.g., marshmallows, life savers, fruit pieces); in the NOR task, the ability to interact with novel enrichment objects serves as positive reinforcement. For all three tasks, data collection can be accomplished in no more than 10 min/task.

Object Retrieval with Detours (ORD):
The device consists of a clear Plexiglas box with one open side that can be mounted to the cage front. The position of the open side can be varied (e.g., left, right). Food treats can be placed a varying locations in the open side (e.g., outside, inside, deep). Our dependent measures include the number of trials completed successfully as well as the type of error made.
Task phases:
Apparatus habituation I--> box in 'forward easy' position; food at edge of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
Apparatus habituation II --> box in 'forward easy' position; food at back of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
"Easy" training --> all easy trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
"Mixed" training --> mixed trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
Probe trial --> all difficult trials; 2 min to retrieve food; run one day

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

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

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

N/A

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Procedure Name

Human Intruder Test (HIT)

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☑

Describe the procedure, giving enough detail so that another individual could carry it out.

This test assesses behavioral responsiveness to both a potentially threatening (direct eye contact from human stranger) and a non-threatening (human stranger present but not making direct eye contact) social stimulus. Monkeys are given a brief acclimation period (10 min; to video camera positioned in front of cage), and are then exposed to two 2-min periods with a human intruder who stands approximately 0.6 m from the cage. In the first period (PROFILE) the intruder stands with their facial profile to the monkey, taking care not to make eye contact with the monkey. In the other period (STARE), the intruder makes continuous direct eye contact with the monkey. Behaviors that are scored during subsequent scoring of the videotapes include vocalizations, movement, and reaction to stranger, including freezing, fearful and threatening expressions.

Immediately after the HIT test and then two weeks later, monkeys will be sedated with ketamine and blood, hair and saliva samples collected for analysis of stress and other hormones.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ◉ No ○

Indicate the body fluid or material to be collected.

rhesus monkey blood, hair, saliva

Indicate the method and site of collection.

Blood will be collected from the femoral or saphenous vein. Saliva will be collected from the mouth/cheek with cotton wicks. Hair will be shaved from the neck area. Typically, monkeys will be lightly sedated with Ketaset (ketamine; 10-20 mg/kg, i.m.) for the collection of all above samples.

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

2-3 mls of blood; enough saliva to dampen a cotton wick; a 2" X 2" patch of hair

Indicate the frequency of collection.

immediately after the human intruder test and then again 2 weeks after

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Procedure Name

Motor function test (mMAP)

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

We assess motor performance using the monkey Movement Assessment Panel. Testing is conducted by trained technicians and occurs in the animal's home cage with the device mounted to the front of the cage. No preliminary training, food or fluid restriction is necessary to induce the animals to perform the tasks. Rather, positive reinforcement is used. Monkeys are rewarded with food treats (e.g., life savers, fruit pieces). Data collection can be accomplished in ~ 10 min/day.

A small food treat is placed in the food receptacle. The monkey is required to reach through portal A and then through portal B to reach the food; photocells measure the time to retrieve the food. Three levels of difficulty will be used: 1) platform (the monkey retrieves food from a flat platform in the food receptacle), 2) straight rod (the monkey retrieves food that has been threaded on a straight metal rod), and 3) curved rod (the monkey retrieves food that has been threaded on a C-curved metal rod).

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

N/A

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Procedure Name

Relapse Procedure

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

Three types of relapse procedures may be used: reinstatement, resurgence, cue-exposure. Details are provided below.

REINSTATEMENT: Oral self-administration behavior initially is extinguished.
During extinction sessions, alcohol-seeking behavior will be extinguished by eliminating the alcohol-paired stimulus lights and discontinuing liquid deliveries. Extinction sessions will continue for 14 days or until responding decreases and stabilizes, with no systematic trends in responding observed from day-to-day. Once behavior decreases, we will begin testing. Using the extinction condition as a baseline, we will use a priming procedure to determine the degree to which alcohol primes reinstate alcohol-seeking behavior. Primes will be administered orally before test sessions in which the alcohol-paired light also is restored around the spout previously associated with alcohol. During tests, session length will be limited to one hour. On test days, we will vary systematically the alcohol priming dose over a full range (0.5 ? 2 g/kg). In addition, on random test days, the "priming" bottle will contain sucrose solution only and serve as a vehicle control condition. To administer the primes, a bottle containing the priming solution (alcohol in a sucrose solution) will be made available 30 min prior to the start of the test session. After 30 min, the "priming" bottle will be removed and the session will begin. At this point, we can begin administering test drugs alone as primes or before priming with alcohol.

RESURGENCE: Oral self-administration behavior is initially extinguished. During extinction sessions, alcohol-seeking behavior will be extinguished under a differential-reinforcement-of-other behavior (DRO) schedule and in the absence of alcohol availability. That is, for every 10-s that the monkey fails to press the alcohol-associated lever, Bioserve flavored pellets will be delivered. Through this provision of alternative reinforcement, monkeys learn to withhold behavior to receive the food pellets. Note that water will be made available during extinction sessions. Extinction sessions will continue until responding decreases and stabilizes, with no systematic trends in responding observed from day-to-day. Once behavior is extinguished we will begin the resurgence phase. In this phase, neither alcohol, water or Bioserve pellets will be delivered (i.e., lever presses will be counted but not result in any programmed consequences). Typically, when delivery of the alternative reinforcer is discontinued, monkeys will begin to exhibit alcohol-seeking behavior (i.e., press the alcohol-associated lever again). This resurgence of behavior is deemed "relapse". During tests, session length will be limited to one hour, and thus monkeys will have no fluid access for 1 hr. Test drugs may be administered during the extinction or resurgence phases depending on the experimental question.

CUE EXPOSURE: Self-administration behavior initially will be extinguished.
Extinction sessions will be conducted by withholding alcohol presentation yet maintaining response-contingent presentations of the alcohol-paired stimulus light. Extinction will continue across days until the number of lever presses decreases and stabilizes. Once behavior is extinguished, monkeys will remain in their home cages for four days before undergoing a cue exposure test. The cue exposure test is a one session re-exposure to extinction conditions (i.e., re-exposure to alcohol-paired cue lights in the absence of alcohol). Test drugs may be administered in the extinction and/or cue-exposure phase, depending on the experimental question.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

N/A

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Procedure Name

self Admistration

Check if this procedure involves collecting Biological Samples from live animals.
For example, blood, urine, ascites, tail tips, cerebrospinal fluid, biopsy, etc.

☐

Describe the procedure, giving enough detail so that another individual could carry it out.

All operant self-administration is conducted in the monkey's home cage. One side of the cage has been modified to accept a drinking panel. The drinking panel is equipped with two sets of stimulus lights, two retractable sippers, two response levers, two stainless steel reservoirs to hold solutions, and a pellet dispenser with associated food hopper. The monkey receives all training via the stages of the experiment (see below). Drinking sessions last 1-3-hr (depending on phase or study) and occur Monday through Friday (and occasionally weekends/holidays, depending on phase or study).

Training: Initially, monkeys will be habituated to the drinking panels and induced to consume water using scheduled food pellet deliveries in 3-hr daily sessions. White stimulus lights located above the spouts in the center of the operant panel will be lit indicating the start of the experimental session and availability of liquids. Water will be available from one spout, and extension of the spout (triggered by depression of the associated response lever) will be signaled by illumination of green spout lights (water-paired lights) for the duration of extension. Food pellets will be delivered to a receptacle located below the spouts in the center of the operant panel at a fixed time interval that has been shown to induce rhesus monkeys to drink available liquid (e.g., every 300 s), including alcohol. One hour prior to the session, the water sipper line will be unhooked from the cage, and it will be replaced one hour after the session (thus water is unavailable 2-hr/day in this phase). We anticipate that the training phase will last a minimum of 2 weeks and will continue until all animals are drinking water from the drinking panel.

Induction: Alcohol self-administration will be induced during 3-hr daily sessions using a procedure identical to that employed during the Training phase. That is, the water sipper line will be unhooked from the cage (to be replaced post session), the white stimulus lights will be lit indicating the start of the experimental session and food pellets will be delivered at the same fixed time interval as was used in training. However, during Induction, alcohol solution will be available from the "non-water" spout, and extension of this spout (triggered by depression of the associated response lever) will be signaled by illumination of red spout lights (alcohol-paired lights) for the duration of extension. From a water scheduling perspective, during this phase, animals will not have access to water for 5-

hr/day (although, alcohol solution is available for 3 of the 5 hrs).

In this phase, the available alcohol volume will increase in a stepwise fashion over a minimum of a 30-day period. Specifically, the volume of 4% w/v alcohol to deliver a 0.5 g/kg dose will be available for ~30 days, followed by the volume of 4% w/v alcohol to deliver a 1 g/kg dose for ~30 days, and ending with the volume of 4% w/v alcohol to deliver a 1.5 g/kg dose will be available for ~30 days.

A similar step-wise approach is used to induce drinking of sucrose solutions and other drug solutions (i.e., opioids, benzodiazepines) if the study calls for it.

At this point, animals are considered to be trained to self-administer alcohol (sucrose or other drugs) and they move to maintenance conditions. Maintenance self-administration sessions will occur daily and last for 3 hrs during which the water sipper line will be unhooked from the cage (1-hr prior to 1-hr post). In addition, food pellet delivery will be discontinued and water and alcohol (or sucrose or other drug solution) will be made available concurrently. This results in 5-hr off the sipper line, but during 3 of the 5 hrs water and alcohol/sucrose/other drug are available to drink via the operant panel. As in the initial two phases, extension of the water spout will be signaled by illumination of green spout lights and extension of the alcohol/sucrose/other drug spout will be signaled by illumination of red spout lights (extension of either spout triggered by depression of the associated levers). Self-administration of alcohol, sucrose, or other drug solution is maintained under a fixed-ratio schedule of oral delivery such that a particular number of lever presses (initially, a single press) results in extension of a fluid spout.

At this point, various manipulations can occur depending on the phase of the study. These manipulations include: altering the concentration of alcohol/sucrose/drug available for self-administration; altering the number of lever press responses required for sipper extension; administering active doses of opioid or GABAergic drugs before the session.

Will the animal(s) be anesthetized or sedated during this procedure?

Yes ○ No ◉

Describe the restraint method. If this method is prolonged restraint, complete the prolonged restraint section.

N/A

Indicate the body fluid or material to be collected.

Indicate the method and site of collection.

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

Indicate the frequency of collection.

Experimental Agents

Non-Pharmaceutical Grade Agents

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 the Drugs section.

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 (http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm)) and veterinary drugs (the Green Book (https://www.fda.gov/animal-veterinary/products/approved-animal-drug-products-green-book)).

Can't find your Agent?

Download the *Cayuse Experimental Agents Instructions* (https://intranet.umc.edu/Research/Research%20Offices/Office%20of%20Animal%20Welfare/Forms.html), complete the table and attach it to your submission.

If none of your experimental agents are available, add "Other" to the table and attach the completed table from the Cayuse Experimental Agents Instructions form.

Class	Category	Agent	Dose	Volume/Frequency	Vehicle	Route of Administration	Pharma Grade?	Haza
	Chemical Hazard	Mu Opioid Agonists	dependent on specific drug (see attached sheet)	dependent on specific drug (see attached sheet)	dependent on specific drug (see attached sheet)	IM;PO	No	

Class	Category	Agent	Dose	Volume/Frequency	Vehicle	Route of Administration	Pharma Grade?	Haza
		Ethanol	Set by the monkey (typically 0-3.5 g/kg daily)	typically <1-3 L of (0.5-32% w/v); up to 5 day's per week	water	PO	Yes	Yes
		Benzodiazepines	dependent on specific drug (see attached sheet)	dependent on specific drug (see attached sheet)	dependent on specific drug (see attached sheet)	IM;PO	No	
		sucrose solution	set by the monkey	< 1-3 L; up to 5 days/week	water	PO	No	
	Chemical Hazard	Naltrexone HCl	0.01 - 0.3 mg/kg	< 0.2 ml/kg	saline	IM	No	

Category

Chemical Hazard

Name of Agent/Material/Compound

Mu Opioid Agonists

Dose

dependent on specific drug (see attached sheet)

Volume and Frequency of Administration

dependent on specific drug (see attached sheet)

Vehicle

dependent on specific drug (see attached sheet)

Route of Administration

IM;PO

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 the opioid agonists (e.g., morphine, oxycodone, fentanyl, alfentanil), no adverse events are expected at the doses, frequencies, and route of administration proposed. Potential side effects of opioid agonists are ataxia, sedation, and at high doses, respiratory depression. Opioids can also cause constipation. In the unlikely event that these indications develop, naltrexone (0.3 mg/kg, i.m., or to effect) will be administered immediately to alleviate the physical symptoms.

Is this a Pharmaceutical Grade Agent?
Link to AAALAC FAQ about Non-Pharmaceutical Grade Compounds (https://www.aaalac.org/accreditation-program/faqs/#B9)

Yes ○ No ◉

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

Our studies require formulations compatible with i.m. administration (i.e., compounds should be in a pure form without additional solvents that may have intrinsic effects [e.g., glycine], and be amenable to dissolution in an acceptable vehicle). All non-pharmaceutical-grade compounds are either purchased from commercial vendors or prepared by medicinal chemists.

Morphine sulfate/fentanyl citrate/alfentanil --> Although these compounds are available in an injectable formulation, the available concentrations are too low and injections at the higher end of our dose ranges would be prohibitively large.

Oxycodone --> No injectable solutions are available.

Buprenorphine ? Buprenorphine is available in multiple formulations. Several (transdermal, tablet, buccal, sublingual) are not appropriate for our usage. An injectable formulation is available, but the concentration is too low and injections at the higher end of our dose range would be prohibitively large.

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

We acquire naltrexone and other drugs with ~99% purity from commercial sources, prepare the solutions with precision instruments (and with trained personnel), and filter-sterilize all solutions with 200 micron filters into

autoclaved containers

Is this a chemical, biological, or radiation hazard?

Yes ○ No ○

Category

Name of Agent/Material/Compound

Ethanol

Dose

Set by the monkey (typically 0-3.5 g/kg daily)

Volume and Frequency of Administration

typically <1-3 L of (0.5-32% w/v); up to 5 day's per week

Vehicle

water

Route of Administration

PO

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.).

Ethanol is a CNS depressant. During the course of our studies, it is possible that subjects could drink enough ethanol such that they would experience some temporary sedation and/or ataxia. The likelihood of this occurring is decreased by limiting the length of access to ethanol to 1-3 hrs. Moreover, monkeys will be observed by trained staff immediately after the self-administration sessions for sedation, ataxia and/or withdrawal signs (tremors, retching, vomiting). Although the experimental conditions of our studies should not result in the development of physical dependence, in the unlikely event that mild withdrawal-like indications develop, diazepam will be administered immediately to alleviate the physical symptoms, and alcohol self-administration will be ended by gradually reducing the availability over a period of weeks to avoid possible precipitation of more severe symptoms. If it is deemed safe to do so, CCR staff will restrain the monkey and administer diazepam IV (1 -3 mg/kg or to effect). If it is not safe to administer diazepam IV, the IM route will be used ((1 - 3 mg/kg, b.i.d., i.m., or to effect).

Is this a Pharmaceutical Grade Agent?
Link to AAALAC FAQ about Non-Pharmaceutical Grade Compounds (https://www.aaalac.org/accreditation-program/faqs/#B9)

Yes ◉ No ○

Is this a chemical, biological, or radiation hazard?

Yes ◉ No ○

Is the Hazardous Use form attached?
The form is required. Please attach the completed form after saving the page.

Yes ◉ No ○

Category

Name of Agent/Material/Compound

Benzodiazepines

Dose

dependent on specific drug (see attached sheet)

Volume and Frequency of Administration

dependent on specific drug (see attached sheet)

Vehicle

dependent on specific drug (see attached sheet)

Route of Administration

IM;PO

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.).

GABA-A benzodiazepine agonists (e.g., diazepam, triazolam, experimental drugs, etc.) could cause temporary and mild sedation and/or ataxia. If deemed hazardous, these effects are reversible with administration of the antagonist flumazenil.

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

GABA-A benzodiazepine antagonists (e.g., flumazenil, experimental drugs, etc.) should have no behavioral effects on their own.

Is this a Pharmaceutical Grade Agent?
Link to AAALAC FAQ about Non-Pharmaceutical Grade Compounds (https://www.aaalac.org/accreditation-program/faqs/#B9)

Yes ○ No ◉

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

Our studies require formulations compatible for i.m./oral administrations. Diazepam, triazolam, and zolpidem are available in pharmaceutical grade, tablet formulations. However, these cannot be dissolved appropriately for preparation of i.m./oral formulations due to the inactive ingredients required to prepare a tablet or capsule formulation. Flumazenil is available in an appropriate formulation (i.e., liquid), but the available concentrations (e.g., 0.1 mg/ml) are too low to meet our needs (i.e., to administer this pharmaceutical grade formulation, too high volumes would be required). Finally, a good number of the compounds we use are experimental in nature. In many cases, veterinary or human pharmaceutical grade products are not available. In others, the compounds are the product of our collaboration with [redacted] a medicinal chemist from the University of Wisconsin-Milwaukee.

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

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

Is this a chemical, biological, or radiation hazard?

Yes ○ No ○

Category

Name of Agent/Material/Compound

sucrose solution

Dose

set by the monkey

Volume and Frequency of Administration

< 1-3 L; up to 5 days/week

Vehicle

water

Route of Administration

PO

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.).

none

Is this a Pharmaceutical Grade Agent?
Link to AAALAC FAQ about Non-Pharmaceutical Grade Compounds (https://www.aaalac.org/accreditation-program/faqs/#B9)

Yes ○ No ◉

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

No veterinary or human pharmaceutical-grade sucrose is available.

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

We purchase human grade sucrose (sugar) at the grocery store.

Is this a chemical, biological, or radiation hazard?

Yes ○ No ○

Category

Chemical Hazard

Name of Agent/Material/Compound

Naltrexone HCl

Dose

0.01 - 0.3 mg/kg

Volume and Frequency of Administration

< 0.2 ml/kg

Vehicle

saline

Route of Administration

IM

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.).

Naltrexone will be administered at doses and concentrations within proven safe parameters in monkeys and is not expected to produce any adverse side effects that would require medical attention or endanger the health of the animal

Is this a Pharmaceutical Grade Agent?
Link to AAALAC FAQ about Non-Pharmaceutical Grade Compounds (https://www.aaalac.org/accreditation-program/faqs/#B9)

Yes ○ No ◉

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

Naltrexone is only available in tablet or extended release formulations; neither of which would meet the need of our studies. For example, tablets/capsules cannot be dissolved appropriately for preparation of i.m. formulations due, in part, to the inactive ingredients.

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

We acquire naltrexone and other drugs with ~99% purity from commercial sources, prepare the solutions with precision instruments (and with trained personnel), and filter-sterilize all solutions with 200 micron filters into autoclaved containers

Is this a chemical, biological, or radiation hazard?

Yes ○ No ○

Euthanasia Method Information

AVMA Guidelines for the Euthanasia of Animals (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)

Euthansia Method	Secondary Method
Ketamine followed by Commercial Euthanasia Agent	It is typical for the CCR veterinary staff to perform a necropsy on all nonhuman primates after euthanasia. The necropsy would constitute a secondary method of euthanasia.

Euthanasia Method

Ketamine followed by Commercial Euthanasia Agent

AVMA Classification

Acceptable

If method is not AVMA acceptable, please provide justification

It is acceptable

State the secondary method of euthanasia or assurance of death.

It is typical for the CCR veterinary staff to perform a necropsy on all nonhuman primates after euthanasia. The necropsy would constitute a secondary method of euthanasia.

Justification of Animal Numbers

USDA Category	# of Animals
B	0
C	0
D	36
E	0

For reference, the USDA Categories are defined
B = animal held for breeding and/or not yet used in research
C = no pain or distress
D = alleviated pain or distress
E = unalleviated pain or distress

Justify the numbers of animals to be used.

- **Describe the statistical method (or other method) used to justify the number of animals per group. Federal guidance states that statistical methods must be used in order to justify the number of animals requested.**
- **Describe mortality or exclusion rates if applicable.**
- **Be sure to include breeding colony numbers (production, maintenance, undesired genotypes).**
- **The numbers provided in this section must also match the total amount in the # of animals column above.**

The number of monkeys to use in *in vivo* pharmacology studies is a decision that involves a trade-off between using large numbers of animals and assuring the reliability of data collected using small numbers of animals. The proposed studies in this application are designed to increase the reliability of data from small numbers of monkeys by using,

whenever possible, a *within-subjects experimental design*. This design, in which each animal serves as its own control, permits scientifically meaningful results to be obtained with fewer animals than would be required with other types of designs (e.g., an exclusively between-subjects approach). For the current protocol, we propose the use of 30 monkeys. Specifically: alcohol self-administration – 24 monkeys (N=6 per sex or genotype); sucrose self-administration – 12 monkeys (N=6 per sex). All other of the above mentioned procedures will be conducted in the same self-administration monkeys.

We have used this approach since the inception of the primary NIH grant in 2006 that supports this research, and the approach has a long history in behavioral pharmacology research dating back to the 1960's. Based on statistical power assessments obtained from analyses of previous data sets, 6 monkeys/sex or genotype are sufficient to draw reliable conclusions from experiments of the type proposed here.

Methodology

For each species, provide a general description of all protocols, aims, study groups, and/or timelines.

Please list the interventions/procedures in chronological order, indicating the time interval between each procedure, and the final disposition of the animals at the end of the experiment. Flowcharts, timelines, or other graphical representations can be included to clarify the timing of procedures. **All procedures listed in the table on the procedures page must be included;** however, the procedural details described elsewhere do not need to be included here.

This text box allows for tables, pictures, and flowcharts to be inserted using the picture icon. It is preferred that all tables, charts, and pictures be inserted in the text box but you may attach the files below if needed. Please contact a member of the IACUC or veterinary staff should you require guidance regarding the information and level of detail that should be provided here.

Brief Outline

Initial behavioral characterization (12 weeks): Upon entry to the lab, monkeys will initially undergo "behavioral profiling". They will be tested in cognitive tests, the Human Intruder test, and the motor function test. Additionally, they will undergo behavioral observation.

Cognitive testing: 6 weeks to complete three different cognitive tasks

Motor function test: 2 weeks

Human Intruder test: 4 weeks for 2 determinations

Behavioral observation: concurrent with the above tests

Self-administration (~12 months to complete all phases): After initial behavioral characterization, monkeys will be trained to orally self-administer a solution (depending on the group and/or experimental question, the solution is either alcohol, sucrose, an opioid agonist, or a benzodiazepine agonist).

Panel training: Initially, monkeys will be habituated to the cage-associated drinking panels and induced to consume water using scheduled food pellet deliveries (these food pellets are above and beyond their daily food ration) in 3-hr daily sessions. We anticipate that the training phase will last a minimum of 2 weeks and will continue until all animals are drinking water from the drinking panel.

Induction: Self-administration will be induced during 3-hr daily sessions using a procedure identical to that employed during the Training phase. However, during Induction, alcohol/drug solution will be available from the "non-water" spout. In this phase, the available alcohol/drug dose will increase in a stepwise fashion over a minimum of a 14-day period

Limited access - maintenance: Following the Induction phase, maintenance self-administration sessions will occur daily and last for 3 hrs during which the water sipper line will be unhooked from the cage. In addition, food pellet delivery will be discontinued and water and alcohol/drug solution will be made available concurrently. This phase will last 30 days.

Limited access - variable: In this phase, we will determine an alcohol/drug concentration-response function. Self-administration sessions will occur as described for Maintenance. The sole difference will be that we will vary systematically the concentration of alcohol/drug that is available for self-administration. Each concentration will be available concurrently with water for a 14- to 30-day period.

**Throughout this 12-month self-administration period, behavioral observation will be conducted concurrently. Activity monitoring also may be conducted. Blood collection will occur in alcohol self-administrating animals to determine blood alcohol levels. Blood draws will occur at key points during self-administration (e.g., at the end of each induction step, on the final stable day at each concentration during determination of a concentration-response function, etc.)

Once monkeys complete the self-administration protocol, their experimental trajectories can diverge. Some will continue in self-administration and undergo pharmacological testing. Others will undergo relapse procedures (which include pharmacological tests).

Pharmacological testing (total time dependent on # of pharmacological agents): Regardless of the self-administered solution, a similar pharmacological testing approach will be used. Pretreatment studies with our experimental agents will be conducted to determine, for example, whether an agonist can enhance the effects of the solution or the degree to which an antagonist can block self-administration of the solution (up to 6 months per pretreatment drug depending on the procedure**)**.

Relapse procedures (total time dependent on the specific procedure and # of pharmacological agents):

*Reinstatement (**~1 year to establish the baseline priming dose-response function and before experimental agents can be introduced**)*: Oral self administration behavior initially is extinguished. During extinction sessions, alcohol-/drug seeking behavior will be extinguished by eliminating the alcohol/drug-paired stimulus lights and discontinuing liquid deliveries. Extinction sessions will continue for 14 days and until responding decreases and stabilizes. Once behavior decreases, we will begin testing. Using the extinction condition as a baseline, we will use a priming procedure to determine the degree to which alcohol/drug primes reinstate alcohol/drug-seeking behavior. Primes will be administered orally before test sessions in which the alcohol/drug-paired light also is restored. During tests, session length will be limited to 1 hour. On test days, we will vary systematically the alcohol/drug priming dose over a full range. In addition, on random test days, the "priming" bottle will contain sucrose solution only and serve as a vehicle control condition. To administer the primes, a bottle containing the priming solution (alcohol/drug in a sucrose solution) will be made available 30 min prior to the start of the test session. After 30 min, the "priming" bottle will be removed and the session will begin. After establishing a priming dose-response function for the training drug, we can begin administering pharmacological agents alone as primes or before priming with the parent drug.

*Resurgence (**~3-4 months for a single resurgence "cycle"; # of cycles depends on pharmacological agent**)*: Oral self administration behavior initially is extinguished. During extinction sessions, alcohol/drug-seeking behavior will be extinguished under a differential-reinforcement-of-other behavior (DRO) schedule and in the absence of alcohol availability. That is, for every 10-s that the monkey fails to press the alcohol/drug-associated lever, flavored food pellets will be delivered. Through this provision of alternative reinforcement, monkeys learn to withhold behavior to receive the food pellets. Note that water will be made available during extinction sessions. Extinction sessions will continue until responding decreases and stabilizes, with no systematic trends in responding observed from day-to-day. Once behavior is extinguished we will begin the resurgence phase. In this phase, neither alcohol/drug, water or pellets will be delivered (i.e., lever presses will be counted but not result in any programmed consequences). Typically, when delivery of the alternative reinforcer is discontinued, monkeys will begin to exhibit alcohol/drug-seeking behavior (i.e., press the alcohol/drug-associated lever again). This resurgence of behavior is deemed "relapse". During tests, session length will be limited to one hour, and thus monkeys will have no fluid access for 1 hr. At this point, we can begin administering active doses of pharmacological agents before the session, in either extinction or resurgence phases.

*Cue Exposure (**~2-3 months for a single cue-exposure "cycle"; # of cycles depends on pharmacological agent**)*: Oral self administration behavior initially is extinguished. During extinction sessions, alcohol/drug presentation is omitted response-contingent presentations of the alcohol/drug-paired stimulus light are maintained. Extinction will continue across days until the number of lever presses decreases and stabilizes. Once behavior is extinguished, monkeys will remain in their home cages for four days before undergoing a cue exposure test. The cue exposure test is a one session re-exposure to extinction conditions (i.e., re-exposure to alcohol/drug-paired cue lights in the absence of alcohol/drug). Assessment of reacquisition of alcohol/drug self-administration will begin the day after the cue exposure test. Reacquisition will use conditions identical to those of baseline alcohol/drug self-administration. Importantly, no alcohol/drug priming or other inducements to initiate lever pressing will be given. At this point, we can begin administering active doses of pharmacological agents before the session, in either extinction or cue exposure phases.

**Throughout these "post-self-administration" procedures, behavioral observation may be conducted periodically, depending on the experimental question. Blood collection also may occur in alcohol self-administrating animals to determine blood alcohol levels. Blood draws will occur at key points during these procedures (e.g., on the final stable day of a specific pretreatment, after a priming dose of alcohol, etc.)

When an animal completes a specific study, it will be enrolled in a new study on this protocol or transferred to a different protocol, pending veterinary approval. Euthanasia is not a part of the proposed experiments and will be performed only as necessary (e.g., due to terminal illness).

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. Explain 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 that incorporate animal manipulations or procedures that 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 (https://documents.umc.edu/PolicyList.aspx?mid=c68315a7-aa84-45c9-96bc-d45680060514&pa=).

Will the animals be subjected to procedures involving more than momentary pain and distress?

Yes ○ No ◉

Behavioral Training and Testing

NIH Publication: *Methods and Welfare Considerations in Behavioral Research with Animals NIH Publication No. 02-5083, March 2002*

https://www.nimh.nih.gov/funding/grant-writing-and-application-process/animals_43007.pdf (https://www.nimh.nih.gov/funding/grant-writing-and-application-process/animals_43007.pdf)

American Physiological Society Publication: *Resource Book for the Design of Animal Exercise Protocols*, Feb. 2006

https://www.physiology.org/docs/default-source/science-policy/animalresearch/resource-book-for-the-design-of-animal-exercise-protocols.pdf?sfvrsn=43d9355b_12 (https://www.physiology.org/docs/default-source/science-policy/animalresearch/resource-book-for-the-design-of-animal-exercise-protocols.pdf?sfvrsn=43d9355b_12)

Will behavioral training or testing be conducted?

Yes (●) No ( )

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

Operant alcohol/drug/sucrose self-administration; cognitive testing; motor function testing; observation of species-typical behavior; activity monitoring

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

Operant alcohol/drug/sucrose self-administration:
All operant self-administration is conducted in the monkey's home cage. One side of the cage has been modified to accept a drinking panel. The drinking panel is equipped with two sets of stimulus lights, two retractable sippers, two response levers, two stainless steel reservoirs to hold solutions, and a pellet dispenser with associated food hopper. The monkey receives all training via the stages of the experiment (see below). Food restriction is not necessary, but we do use water scheduling as described below. Drinking sessions last 1-3-hr (depending on phase) and occur Monday through Friday (and occasionally weekends/holidays, depending on phase).

Training: Initially, monkeys will be habituated to the drinking panels and induced to consume water using scheduled food pellet deliveries in 3-hr daily sessions. White stimulus lights located above the spouts in the center of the operant panel will be lit indicating the start of the experimental session and availability of liquids. Water will be available from one spout, and extension of the spout (triggered by depression of the associated response lever) will be signaled by illumination of green spout lights (water-paired lights) for the duration of extension. Food pellets will be delivered to a receptacle located below the spouts in the center of the operant panel at a fixed time interval that has been shown to induce rhesus monkeys to drink available liquid (e.g., every 300 s). One hour prior to the session, the water sipper line will be unhooked from the cage, and it will be replaced one hour after the session (thus water is unavailable 2-hr/day in this phase). We anticipate that the training phase will last a minimum of 2 weeks and will continue until all animals are drinking water from the drinking panel.

Induction: Alcohol/drug/sucrose self-administration will be induced during 3-hr daily sessions using a procedure identical to that employed during the Training phase. That is, the water sipper line will be unhooked from the cage (to be replaced post session), the white stimulus lights will be lit indicating the start of the experimental session and food pellets will be delivered at the same fixed time interval as was used in training. However, during Induction, alcohol/drug/sucrose solution will be available from the "non-water" spout, and extension of this spout (triggered by depression of the associated response lever) will be signaled by illumination of red spout lights (alcohol/drug/sucrose-paired lights) for the duration of extension. From a water scheduling perspective, during this phase, animals will not have access to water for 5-hr/day (although, another solution is available for 3 of the 5 hrs).

In this phase, the available alcohol/drug/sucrose volume will increase in a stepwise fashion over a minimum of a 30-day period to slowly habituate the monkey to the pharmacological effects of the solution. In alcohol self-administering animals, on the final day of testing at each concentration, blood will be drawn and BALs will be determined.

At this point, animals are considered to be trained to self-administer alcohol/drug/sucrose and they move to maintenance conditions. Maintenance self-administration sessions will occur daily and last for 3 hrs during which the water sipper line will be unhooked from the cage (1-hr prior to 1-hr post). In addition, food pellet delivery will be discontinued and water and alcohol/drug/sucrose will be made available concurrently. This results in 5-hr off the sipper line, but during 3 of the 5 hrs water and alcohol/drug/sucrose are available to drink via the operant panel. As in the initial two phases, extension of the water spout will be signaled by illumination of green spout lights and extension of the alcohol/drug/sucrose spout will be signaled by illumination of red spout lights (extension of either spout triggered by depression of the associated levers). Self-administration of the solutions is maintained under a fixed-ratio schedule of oral solution delivery such that a particular number of lever presses (initially, a single press) results in extension of a fluid spout. After a period of time at maintenance conditions, solution concentrations will be varied to obtain full concentration-response functions. At this point, experimental trajectories will diverge and could include testing of pharmacological agents and/or entering a relapse procedure (see below).

Relapse - reinstatement:
Once self-administration is established, behavior will be extinguished. During extinction sessions, alcohol/drug-seeking behavior will be extinguished by eliminating the alcohol/drug-paired stimulus lights and discontinuing liquid deliveries. Extinction sessions will continue for 14 days or until responding decreases and stabilizes, with no systematic trends in responding observed from day-to-day. Once behavior decreases, we will begin testing. Using the extinction condition as a baseline, we will use a priming procedure to determine the degree to which alcohol/drug primes reinstate alcohol/drug-seeking behavior. Primes will be administered orally before test sessions in which the alcohol/drug-paired light also is restored. During tests, session length will be limited to one hour. On test days, we will vary systematically the alcohol/drug priming dose over a full range. In addition, on random test days, the "priming" bottle will contain sucrose solution only and serve as a vehicle control condition. To administer the primes, a bottle containing the priming solution (alcohol/drug in a sucrose solution) will be made available 30 min prior to the start of the test session. After 30 min, the "priming" bottle will be removed and the session will begin. At this point, we can begin administering test drugs alone as primes or before priming with alcohol/drug.

Relapse - resurgence:
Once animals complete the self-administration protocol, their behavior will be extinguished. During extinction sessions, alcohol/drug/sucrose-seeking behavior will be extinguished under a differential-reinforcement-of-other behavior (DRO) schedule and in the absence of alcohol/drug/sucrose availability. That is, for every 10-s that the monkey fails to press the alcohol/drug/sucrose-associated lever, flavored pellets will be delivered. Through this provision of alternative reinforcement, monkeys learn to withhold behavior to receive the food pellets. Note that water will be made available during extinction sessions. Extinction sessions will continue until responding decreases and stabilizes, with no systematic trends in responding observed from day-to-day. Once behavior is extinguished we will begin the resurgence phase. In this phase, neither alcohol/drug/sucrose, water or pellets will

be delivered (i.e., lever presses will be counted but not result in any programmed consequences). Typically, when delivery of the alternative reinforcer is discontinued, monkeys will begin to exhibit alcohol/drug/sucrose-seeking behavior (i.e., press the alcohol/drug/sucrose-associated lever again). This resurgence of behavior is deemed "relapse". During tests, session length will be limited to one hour, and thus monkeys will have no fluid access for 1 hr. At this point, we can begin administering active doses of experimental agents before the session, in either extinction or resurgence phases.

Relapse - cue exposure:
Once self-administration behavior is established, it will be extinguished. Extinction sessions will be conducted by withholding alcohol/drug presentation yet maintaining response-contingent presentations of the alcohol/drug-paired stimulus light. Extinction will continue across days until the number of lever presses decreases and stabilizes. Once behavior is extinguished, monkeys will remain in their home cages for four days before undergoing a cue exposure test. The cue exposure test is a one session re-exposure to extinction conditions (i.e., re-exposure to alcohol/drug-paired cue lights in the absence of alcohol/drug). Assessment of reacquisition of alcohol/drug self-administration will begin the day after the cue exposure test. Reacquisition will use conditions identical to those of baseline alcohol/drug self-administration. Importantly, no alcohol/drug priming or other inducements to initiate lever pressing will be given.

Administration of experimental agents in all studies:
Pretreatments will be administered intramuscularly. We employ positive reinforcement techniques (i.e., administration of small food rewards) to accustom the monkeys to all injections and, in our experience, most monkeys come to present a limb or their rump for injections.

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

Object Retrieval with Detours (ORD):
The device consists of a clear Plexiglas box with one open side that can be mounted to the cage front. The position of the open side can be varied (e.g., left, right). Food treats can be placed a varying locations in the open side (e.g., outside, inside, deep). Our dependent measures include the number of trials completed successfully as well as the type of error made.
Task phases:
Apparatus habituation I: box in 'forward easy' position; food at edge of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
Apparatus habituation II: box in 'forward easy' position; food at back of box; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
"Easy" training: all easy trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
"Mixed" training: mixed trials; 2 min to retrieve food; run until monkey retrieves 12/15 treats for 2 days in a row
Probe trial: all difficult trials; 2 min to retrieve food; run one day

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

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

Motor function testing (mMAP): We assess motor performance using the monkey Movement Assessment Panel. Testing is conducted by trained technicians and occurs in the animal's home cage with the device mounted to the front of the cage. No preliminary training, food or fluid restriction is necessary to induce the animals to perform the tasks. Rather, positive reinforcement is used. Monkeys are rewarded with food treats (e.g., life savers, fruit pieces). Data collection can be accomplished in ~ 10 min/day and it will take 3 weeks to complete all phases of the task. A small food treat is placed in the food receptacle. The monkey is required to reach through portal A and then through portal B to reach the food; photocells measure the time to retrieve the food. Three levels of difficulty will be used: 1) platform (the monkey retrieves food from a flat platform in the food receptacle), 2) straight rod (the monkey retrieves food that has been threaded on a straight metal rod), and 3) curved rod (the monkey retrieves food that has been threaded on a C-curved metal rod).

Species-typical observable behavior:
Trained observers will monitor the animals' behavior in order to establish quantitative profiles for behavioral effects in the absence of alcohol/drug, as well as after alcohol/drug self-administration. No devices, training of the animals, food/fluid restriction or positive reinforcement are required. Behavior will be assessed using a modified frequency measure for each animal as described previously by [redacted] et al. (2002; Psychopharmacology 164: 151-159). A range of species typical behaviors, as well as behaviors associated specifically with alcohol, will be recorded during 20 15-second intervals by trained observers (total of 5 min). Observers will be trained using a standard procedures manual and will complete at least 20 hrs of scoring prior to participating in the study. Percent agreement scores will be used to determine inter-observer reliability, with a criterion of = 90% required.

Using this scoring system, the presence of a behavior is noted during each 15-second interval and the number of 15-second intervals during the 5 minute session in which this behavior is observed are recorded. Thus, the maximum score for each behavior during one modified frequency sample is 20. This modified frequency scoring system controls for variability between animals by limiting the number of intervals in which a behavior can occur. Behaviors that will be scored are divided into two different types: Species-typical behavior and study-specific behaviors. Species-typical behaviors are based on observations of monkeys in naturalistic settings and will provide a quantitative analysis of the effects of genotype and/or alcohol on normal behavioral profiles. Study-specific behaviors will be characteristic alcohol/drug-induced effects, including different measures of sedation.

We have developed a scoring system for sedation based on standards used for anesthesia of human patients used by the American Society of Anesthesiologists (ASA 2002). The ASA standards are based on responsiveness to verbal or tactile stimulation as a measure of the degree of sedation. For example, minimal sedation is defined as normal response from the patient following verbal stimulation by a health care specialist, while deep sedation is noted when the patient fails to respond to normal verbal or tactile responses (i.e., the patient requires repeated or painful stimuli to be roused). We have taken these methods and adapted them to rhesus monkeys, with the intent of providing a translational scoring system of drug-induced sedative effects. Two additional categories have been included in the behavior scoring session: Moderate sedation and deep sedation. The species-typical category of sleep posture also is included in the sedation assessment.

In order to evaluate the ability to be roused, we have included an evaluation of responding to external stimuli during the modified frequency scoring session. The evaluation of responsiveness is conducted at the beginning of each 60-second block of time during the scoring session (i.e., maximum of 5 evaluations during one session). If sleep posture was observed and the animal does not attend to the activities of other monkeys in the room, the observer will speak the monkey's name in a normal tone, walk at a normal pace towards the cage, and then move the lock used to secure the door of the cage (a feature present on all cages). If the monkey opens its eyes and initiates movement in less than 3 sec, the observer will stop the evaluation and note "responds readily". If the monkey attends slowly (i.e., = 3 seconds following stimuli) the observer records "delayed response" which is part of a decision rule for "moderate sedation". If the monkey does not respond to these three stimuli, the observer records "no response", which is part of a decision rule for "deep sedation".

Activity monitoring:
Activity will be monitored noninvasively using Actiwatches. Actiwatches are small, lightweight accelerometers that quantify and record movements. They will be placed in a protective case attached to commercially available nonhuman primate collars. The collar will be fitted on the animal under light ketamine anesthesia. This method has been used extensively to quantify activity in nonhuman primates over extended periods of time with no adverse effects. The collar and Actiwatch case do not impede the animal's movement in any manner. Both collar and case are typically well-tolerated by monkeys. Animals will remain in their home cages and no other changes will be made.

For Actiwatch maintenance, the animal will be lightly sedated and the Actiwatch removed in order to download data, change batteries, and reprogram. Actiwatches can record up to 180 days, depending on data collection settings, without needing to be removed. We expect Actiwatches will remain in place up to a maximum of 180 days between changes or removals, although they may be removed earlier for health (e.g. skin irritation beneath the collar), equipment (e.g. loosening of screws holding the Actiwatch case to the collar), or experimental (e.g., cessation of data recording) reasons.

Human Intruder Test (HIT):
This test assesses behavioral responsiveness to both a potentially threatening (direct eye contact from human stranger) and a non-threatening (human stranger present but not making direct eye contact) social stimulus. Monkeys are given a brief acclimation period (10 min; to video camera positioned in front of cage), and are then exposed to two 2-min periods with a human intruder who stands approximately 0.6 m from the cage. In the first period (PROFILE) the intruder stands with their facial profile to the monkey, taking care not to make eye contact with the monkey. In the other period (STARE), the intruder makes continuous direct eye contact with the monkey. Behaviors that are scored during subsequent scoring of the videotapes include vocalizations, movement, and reaction to stranger, including freezing, fearful and threatening expressions. Two weeks later, the HIT will occur again. Immediately after both tests will be sedated with ketamine and blood, hair and saliva samples collected for analysis of stress and other hormones.

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?

During the course of our studies, it is possible that subjects could drink enough ethanol such that they would experience some temporary sedation and/or ataxia. We will minimize the likelihood of this occurring by limiting the length of access to ethanol to 1-3 hrs. Animals will be observed by trained staff immediately after the self-administration sessions for sedation, ataxia and/or withdrawal signs (tremors, retching, vomiting).

Although the experimental conditions of our studies should not result in the development of physical dependence, in the unlikely event that mild withdrawal-like indications develop, diazepam (1 ? 3 mg/kg, b.i.d., i.m., or to effect) will be administered immediately to alleviate the physical symptoms, and alcohol self-administration will be ended by gradually reducing the dosage over a period of weeks to avoid possible precipitation of more severe symptoms. A CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

The administration of GABA-A/benzodiazepine agonists could cause temporary and mild sedation and/or ataxia. Animals will be observed routinely by laboratory staff after the sessions for sedation and/or ataxia. These effects are reversible with administration of the benzodiazepine antagonist flumazenil.

At doses above those proposed for use in our studies, the administration of GABA-A/benzodiazepine inverse agonists can be proconvulsant. Animals will be observed routinely by laboratory staff during and after the experimental session for any signs of convulsant activity. These effects are reversible with administration of diazepam. A CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

Opioid agonists are associated with side effects, including respiratory depression. In the unlikely event that respiratory depression is noted, the opioid antagonist naltrexone (0.03-0.3 mg/kg, i.m., or to effect) will be administered immediately. A CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

Will animal be observed/attended through the duration of the trial/test?

Yes ○ No ◉

Provide scientific rationale for leaving the animal unattended.

In self-administration and relapse studies, we are measuring the behavior of alcohol/drug/sucrose-taking/-seeking. The presence of an observer would change the behavior and add an uncontrolled variable to the experiment. Additionally, self-administration sessions last for 1-3 hours and the preparation is relatively safe. Importantly, animals are observed immediately post session by trained observers.

For all other experimental procedures, an observer is present at all times.

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 the animals on the Rod Test, etc.).

None

Will the Animal Behavior Core (ABC) be used for this testing? If so, attach a copy of the SOPs and Core Director approval.

Yes ○ No ◉

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

[redacted]: 30+ years of experience conducting NHP research of the type described herein

[redacted]: 25+ years of experience conducting NHP research of the type described herein

Exceptions

Will Animals be subject to Food and/or Water Restriction?

Yes ○ No ◉

Will social animals be singly housed?

Yes ◉ No ○

Will any nonstandard husbandry be performed under this protocol? (e.g., delayed weaning, nonstandard space requirements, nonstandard feed, nonstandard water, nonstandard caging, nonstandard room/environment, altered light cycle)

Yes ◉ No ○

Will any expired pharmaceuticals and/or medical materials (e.g., drugs, antibiotics, fluids, saline bags, disinfectant solutions, catheters, sutures, etc.) be used?

Yes ○ No ◉

Are you requesting any exceptions to the guide or to IACUC policies not described elsewhere in the protocol (e.g., group housing over the weight limit, etc.)?

Yes ○ No ◉

Food and Fluid Restriction

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 (https://documents.umc.edu/policy/R-RC-AC-ACG-PO-00001/).

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.

What will be scheduled or restricted?

Select one

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

Restriction protocols typically base the restriction amount relative to a baseline (free-choice consumption) and parameter (body weight, intake amount).
- What will this restriction amount use as the baseline?
- What is the maximum restriction for any animal at one time *(e.g., 5 continuous hours after which animals will have unrestricted access to food/water for the remainder of the day)*?

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?

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

How long will animals be on the regulation protocol?

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

Who will be responsible for administering and documenting the regulation?

Please describe the plan for single housing and provide a scientific justification.

The studies are conducted in monkeys in their individual living quarters. For these studies, the cages have been modified so that experimental equipment can be mounted to a side. Monkeys are trained to self-administer alcohol/drug/sucrose. The effects of alcohol/drug will often persist for several hours, varying with dose and/or concentration tested. The presence of other animals in the living quarters of the experimental subject would interfere with the performance of the animal in the tasks, or alter dramatically the behavior of the monkey during observation sessions, thereby compromising the validity of the research. In all cases, however, individual cages are grouped together in colony rooms in order to allow visual, auditory, and olfactory contact with other monkeys. Tactile contact between cages is also available depending on the compatibility of individual monkeys.

Feeding

Standard

Watering

Nonstandard

Caging

Standard

Room/Environment

Standard

Altered light cycle

Standard

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 nonstandard husbandry must provide complete details of the cleaning and sanitation, especially identifying responsible parties.

Justification for single housed animals made on previous tab.

Watering: Monkeys will undergo water scheduling by personnel listed on the protocol Monday through Friday (not including holidays). Typically, water availability will not be scheduled on weekends and holidays. On the rare occasion when water scheduling must occur on weekends/holidays for experimental reasons, personnel associated with this protocol will be responsible. Induction of alcohol/drug/sucrose self-administration and maintenance of self-administration is facilitated by removing access to water one hour before the start of the session and throughout the session. Access to water is restored one hour after completion of the session (for a total of 5 hours without water; however, during 3 of these hours there is alcohol/drug/sucrose availability, and depending on the experimental phase, water may also be available). All animals assigned to this protocol are observed daily, 5 days per week or more as needed, at least prior to and after their experimental sessions by listed personnel. We monitor weights/physical condition regularly (see below). Fluid intake (in the context of the self-administration experiment) is monitored and recorded each experimental session (typically Monday through Friday, excluding holidays).

Physical exams (includes: determination of weight and body temperature; hands-on assessment of body condition; examination of eyes/ears/teeth/tongue/lips for lesions associated with herpes B virus, etc.; hands-on palpation of abdomen for presence of masses; hands-on examination of pretreatment drug injection site for adverse consequences) will be conducted typically every month (or more frequently, if deemed necessary) by listed personnel. Monkeys will be lightly sedated with ketamine (10-20 mg/kg, i.m.) for the examinations. Physical examinations are conducted for several reasons: the potential for early detection of indicators of health problems (e.g., dehydration, insufficient feed, parasitism, etc.); to obtain recent accurate weights necessary for calculation of doses for pretreatment drugs and alcohol/drug consumption

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'.

Please provide a justification for the use of expired items.

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

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

Please describe the exception and provide justification

Personnel List

All listed personnel must complete IACUC required training, including completion of Occupational Health forms and submit a Training Requirements Registration form (https://intranet.umc.edu/Research/Research%20Offices/Office%20of%20Animal%20Welfare/Forms.html) prior to working with animals and receiving access into the Center for Comparative Research (CCR).

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

Name	Business Role	Phone	Email	Organization	Department	Primary?	Requeste
[redacted]	Principal Investigator	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	Yes	No
[redacted]	Assistant Professor	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Assistant Professor	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Graduate Student		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Researcher 1, 2, or 3		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted] K	Professor	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Researcher 1, 2, or 3		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Researcher 1, 2, or 3	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Researcher 1, 2, or 3	[redacted]	[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Graduate Student		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Researcher 1, 2, or 3		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No
[redacted]	Post Doc		[redacted]	University Of Mississippi Medical Center	Psychiatry and Human Behavior	No	No

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Principal Investigator

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

[redacted]

Emergency Phone

[redacted]

Home Phone

[redacted]

Pager

Primary Contact?

☑

Copy Primary Contact on all Emails

☑

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Euthanasia	Ketamine followed by Commercial Euthanasia Agent
Rhesus Macaque	Procedures	Activity Monitoring
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Blood Collection
Rhesus Macaque	Procedures	Cognitive testing
Rhesus Macaque	Procedures	Human Intruder Test (HIT)
Rhesus Macaque	Procedures	Motor function test (mMAP)
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BA, MS, PhD

Experience and Qualifications

[redacted] has a PhD in Zoology with a Primatology emphasis and over 30 years of experience conducting behavioral and pharmacological research with NHPs. She developed the research program contained in this protocol. She has experience with all procedures involved in running this lab.

- *To add a person, begin typing the last name and the system will suggest registered users.*
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Name

[redacted]

Business Role

Assistant Professor

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

Copy Primary Contact on all Emails

☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Activity Monitoring
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

PhD

Experience and Qualifications

[redacted] has over 5 years of experience conducting behavioral/pharmacological experiments with NHPs. She has been trained on all relevant procedures by PI [redacted] and other lab personnel

---

- *To add a person, begin typing the last name and the system will suggest registered users.*
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Name

[redacted]

Business Role

Assistant Professor

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

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☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

PhD

Experience and Qualifications

[redacted] has a PhD in Psychology and over 15 years of experience conducting behavioral and pharmacological research with rodents and NHPs, including experience with procedures involved in this protocol. She has been trained on all relevant procedures by [redacted]

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Graduate Student

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

Cell Phone

[redacted]

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

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☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BA

Experience and Qualifications

[redacted] has 4+ years of experience with rodents & NHPs . He is a graduate student with [redacted] He has been trained by [redacted] on relevant procedures.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Researcher 1, 2, or 3

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

Copy Primary Contact on all Emails

Will person be handling animal species?

Yes No

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

Experience and Qualifications

[redacted] has over 2 years conducting NHP research in the [redacted] laboratory. He has been trained on all relevant procedures by PIs [redacted] and [redacted] and other lab personnel

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Professor

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

[redacted]
Emergency Phone

[redacted]
Home Phone

[redacted]
Pager

Primary Contact?

Copy Primary Contact on all Emails

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Procedures	Activity Monitoring

Degrees

PhD

Experience and Qualifications

[redacted] has a PhD in Psychology and over 25 years of experience conducting behavioral and pharmacological research with NHPs, including experience with procedures involved in this protocol.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]
Business Role

Researcher 1, 2, or 3

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]
Office Phone

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

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Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BS

Experience and Qualifications

[redacted] has worked for 1+ years in the [redacted] labs. She has been trained on relevant procedures by PIs [redacted] & [redacted] or other lab personnel

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Researcher 1, 2, or 3

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

Copy Primary Contact on all Emails

☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BA in Biochemistry, minor in Biology, University of Mississippi

Experience and Qualifications

No prior experience.

[redacted] and [redacted] and [redacted] will train [redacted] in all monkey-related procedures, including behavioral observations. PPE usage, drug mixing, equipment set-up, computer programming, drug administration, etc. [redacted] and [redacted] have worked with monkeys conducting similar studies to those that [redacted] will run for more than 4 years, and [redacted] has been fully trained to run these studies since she joined our lab in August, 2021.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Researcher 1, 2, or 3

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

[redacted]

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

Copy Primary Contact on all Emails

Will person be handling animal species?

Yes No

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BS in Psychology, University of Southern Mississippi

Experience and Qualifications

None. [redacted] and [redacted] will train [redacted] in all monkey-related procedures, including behavioral observations, PPE usage, drug mixing, equipment set-up, computer programming, drug administration, etc. [redacted] is PI and has conducted these studies for ~20 years. [redacted] and [redacted] have worked with monkeys conducting similar studies to those that [redacted] will run for more than 4 years, and [redacted] has been fully trained to run these studies since she joined our lab in August, 2021.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Graduate Student

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

Copy Primary Contact on all Emails

☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Cognitive testing
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BA

Experience and Qualifications

[redacted] has < 1 year experience in the [redacted] labs, but does come into the lab with ~2 y of prior NHP experience. [redacted] has been trained in relevant procedures by [redacted] [redacted] or other lab personnel.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Researcher 1, 2, or 3

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

☐

Copy Primary Contact on all Emails

☐

Will person be handling animal species?

Yes ◉ No ○

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation
Rhesus Macaque	Procedures	Relapse Procedure
Rhesus Macaque	Procedures	self Admistration

Degrees

BA, MS

Experience and Qualifications

[redacted] has spent 1+ years working in the [redacted] labs. She has been trained in all relevant procedures by [redacted] or other lab personnel.

- *To add a person, begin typing the last name and the system will suggest registered users.*
- *Select the user when the name appears.*
- *If the user is not available, contact the IACUC office to register the new user.*

Name

[redacted]

Business Role

Post Doc

Organization Department

University Of Mississippi Medical Center Psychiatry and Human Behavior

Email

[redacted]

Office Phone

Cell Phone

Emergency Phone

Home Phone

Pager

Primary Contact?

Copy Primary Contact on all Emails

Will person be handling animal species?

Yes No

Species Name	Type	Procedure Description
Rhesus Macaque	Agents	Benzodiazepines
Rhesus Macaque	Agents	Ethanol
Rhesus Macaque	Agents	Mu Opioid Agonists
Rhesus Macaque	Agents	Naltrexone HCl
Rhesus Macaque	Agents	sucrose solution
Rhesus Macaque	Procedures	Behavioral Observation

Degrees

MD

Experience and Qualifications

[redacted] is a post-doctoral fellow in the [redacted] lab. He has < 6 months prior experience working with monkeys in the PIs laboratory. He will be trained in all relevant procedures y the PI or by members of the PI's lab

Databases Searched and 3Rs

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.

Additional assistance may be obtained by contacting the Rowland Medical Library reference desk at ext. [redacted]

List each potentially painful or distressing procedure included in this protocol.

alcohol dependence

opioid dependence

water scheduling

Check a minimum of 2 databases.
Please note: PubMed and Medline are the same and cannot both be used.

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. Specific procedures listed may be utilized as key terms.

Medline/PubMed (http://www.ncbi.nlm.nih.gov/pubmed) (http://www.ncbi.nlm.nih.gov/pubmed)

☑

Toxnet (https://www.nlm.nih.gov/toxnet/index.html) (https://www.nlm.nih.gov/toxnet/index.html)

☐

AWIC (https://www.nal.usda.gov/awic/alternatives-literature-searching) (https://www.nal.usda.gov/awic/alternatives-literature-searching)

☐

Agricola (http://agricola.nal.usda.gov) (http://agricola.nal.usda.gov)

☐

Scopus (http://www.scopus.com/home.url) (http://www.scopus.com/home.url)

☑

Other Database

☐

Paste Literature Search Table Below
Link to Forms Page
(https://intranet.umc.edu/Research/Research%20Offices/Office%20of%20Animal%20Welfare/Forms.html)

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed: Replacement of animals	Reduction in numbers of animals used	Refinement to minimize pain or distress	Lack of unnecessary duplication
Medline/Pubmed	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + self-administration + rhesus monkey + alcohol		x		x
Medline/Pubmed	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + dependence + rhesus monkey + alcohol		x		x
Medline/Pubmed	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + relapse + rhesus monkey + alcohol		x		x

Medline/Pubmed	5/20/22	All searchable years	Alcohol withdrawal	Alcohol use disorders + withdrawal + rhesus monkey + alcohol		x	x	x
Medline/Pubmed	5/20/22	All searchable years	Alcohol dependence/withdrawal	Rhesus monkey + self-administration + alternative + animal model + alcohol	x		x	
Medline/Pubmed	5/20/22	All searchable years	Alcohol dependence/withdrawal	Rhesus monkey + self-administration + in vitro + alcohol	x			
Medline/Pubmed	5/20/22	All searchable years	Water scheduling	Rhesus monkey + water scheduling + alternative			x	
Medline/Pubmed	5/20/22	All searchable years	Opioid withdrawal	Opioid use disorders + withdrawal + rhesus monkey + opioid		x	x	x
Medline/Pubmed	5/20/22	All searchable years	Opioid dependence/withdrawal	Rhesus monkey + self-administration + alternative + animal model + opioid	x		x	
Medline/Pubmed	5/20/22	All searchable years	Opioid dependence/withdrawal	Rhesus monkey + self-administration + in vitro + opioid	x			
Scopus	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + self-administration + rhesus monkey + alcohol		x		x
Scopus	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + dependence + rhesus monkey + alcohol		x		x
Scopus	5/20/22	All searchable years	Alcohol dependence	Alcohol use disorders + relapse + rhesus monkey + alcohol		x		x
Scopus	5/20/22	All searchable years	Alcohol withdrawal	Alcohol use disorders + withdrawal + rhesus monkey + alcohol		x	x	x
Scopus	5/20/22	All searchable years	Alcohol dependence/withdrawal	Rhesus monkey + self-administration + alternative + animal model + alcohol	x		x	

Scopus	5/20/22	All searchable years	Alcohol dependence/withdrawal	Rhesus monkey + self-administration + in vitro + alcohol	x			
Scopus	5/20/22	All searchable years	Water scheduling	Rhesus monkey + water scheduling + alternative			x	
Scopus	5/20/22	All searchable years	Opioid withdrawal	Opioid use disorders + withdrawal + rhesus monkey + opioid		x	x	x
Scopus	5/20/22	All searchable years	Opioid dependence/withdrawal	Rhesus monkey + self-administration + alternative + animal model + opioid	x		x	
Scopus	5/20/22	All searchable years	Opioid dependence/withdrawal	Rhesus monkey + self-administration + in vitro + opioid	x			

Provide a brief summary of any articles that were identified in the search and how these studies relate to the current animal protocol.

The literature searches described above indicate that addiction researchers are using rhesus monkey models of alcohol reinforcement (i.e., self-administration) to investigate pharmacotherapies for the treatment of alcohol use disorders. In contrast, no articles were found detailing the use of rhesus monkeys for alcohol relapse studies indicating that our reinstatement, resurgence and cue exposure studies are not duplicative. Alcohol is known to modulate the GABA-A and opioid systems, so one such strategy for pharmacotherapy development is a focus on drugs that target or interact with GABA-A or opioid receptor subtypes. The hope is that by using subtype-selective compounds, one could identify drugs with anti-alcohol effects yet without untoward side effects such as sedation. Few researchers are taking this approach with rhesus monkey models of the abuse-related effects of alcohol.

In current searches, the proposed methods (e.g., oral self-administration & relapse) are the most commonly used in both rodents and NHPs. Moreover, these methods mimic the route of administration used by humans. There were no other alternatives to the proposed procedures and the work is not unnecessarily duplicative. Alcohol self-administration studies (as well as other operant/observable behavior studies) require intact, behaving organisms. Search hits that included in vitro work were from studies that used these techniques to supplement behavior data, not to replace it.

REPLACEMENT of animals with non-animal techniques (e.g., using tissue culture, computer simulations, etc.).

Please describe why non animal models or invertebrate models will not suffice for your study.

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

REFINEMENT of animal use such as modifying manipulations or measurement techniques to reduce the pain and/or distress experienced by the animals as compared to prior techniques, or using less sentient species (e.g., frog instead of a mouse, mouse instead of dog).

Please describe why less invasive procedures or procedures which may cause less pain or distress cannot be used.

Across all procedures, doses of alcohol and other drugs have been chosen to be those with the least likelihood of producing dependence, withdrawal, and other adverse side effects in subjects. In alcohol/drug self-administration studies specifically, we also have selected experimental parameters (e.g., limited session duration) to reduce the

likelihood of observing dependence and withdrawal. Importantly, we have included monitoring/behavioral scoring periods by technicians trained to recognize species-typical and drug-induced behaviors with the goal of identifying as early as possible side effects that may require intervention (e.g., withdrawal symptoms). Should mild withdrawal-like indications develop, appropriate drugs will be administered immediately to alleviate the physical symptoms, and self-administration will be ended by gradually reducing the dosage over a period of weeks to avoid possible precipitation of more severe symptoms. CCR veterinarians will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

One aspect of our research is the evaluation of inverse agonists as potential alcohol treatments. In general, with these compounds, there is the possibility that they could induce seizures; although, our focus is on subtype-selective compounds where seizure activity is expected to be much less. Nevertheless, we guard against this possibility in several ways: 1) technicians are informed of the possibility of seizures and monitor the animal more frequently (e.g., in addition to the behavioral scoring periods mentioned above) and earlier in the experimental session (e.g., upon start of the session), 2) if pre-seizure activity (e.g., tremors) is noted, testing with that dose is discontinued in the particular animal and not evaluated in any other animal, 3) in the event of a seizure, the monkey will immediately be administered diazepam until seizures abate. A CCR veterinarian will be contacted to provide additional assessment and consultation, as well as additional treatment if needed.

In self-administration studies, a majority of pretreatment drugs are administered intramuscularly. We have alternative strategies in place if daily injections prove to be problematic (e.g., administer orally in jello or other flavorant). We also utilize positive reinforcement techniques to accustom animals to receiving intramuscular injections.

REDUCTION of animal use (i.e., modifying the experimental paradigm or performing statistical analysis to allow the use of fewer animals to obtain the needed information).

Please state how the number of animals you have requested is the minimum needed in order to obtain valid scientific conclusions.

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

Research Endpoints/Chair Approval

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, ex[redacted] for recommendations on the assessment criteria.

Euthanasia is not a part of the proposed experiments and will be performed only as necessary (e.g., due to terminal illness) and in consultation with CCR staff. Reductions in eating and drinking over a 2-day period, a >10% decrease in body weight above and beyond healthy, experimental weight as determined in conjunction with the veterinary staff, and evidence of discomfort, pain or distress (lethargy, abnormal postures, abnormal vocalizations, focus on particular part of body [e.g., limb]) are used as considerations for determination of pain and distress. These behaviors are atypical for the compounds to be evaluated in this protocol. If any of these criteria are observed, a veterinarian from the CCR will be contacted to assess the animal and determine if additional treatment is needed.

At the end of proposed studies, monkeys will be used in subsequent experiments under the same or even other protocols, if deemed suitable by CCR veterinary staff.

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

Yes ○ No ◉

Check to confirm that you have attached the required chair signature form.
If it is not attached, please attach the completed form after saving the page.
Link to the IACUC Forms site
(https://intranet.umc.edu/Research/Research%20Offices/Office%20of%20Animal%20Welfare/Forms.html)

☑

Protocol Attachments

The following is a list of all attachments listed on this Protocol

Page	File Name	Description	Original File Name
Protocol Attachments	2022-1199_1_0001_[redacted] Appendix K.docx (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] Appendix K.docx)	Appendix K - progress report	[redacted] Appendix K.docx
Protocol Attachments	2022-1199_1_0001_[redacted] 1387B IACUC transmittal form - signed.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] 1387B IACUC transmittal form - signed.pdf)	[redacted] - transmittal form - signed by Chair	[redacted] 1387B IACUC transmittal form - signed.pdf
Protocol Attachments	2022-1199_1_0001_SDS - alfentanil.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - alfentanil.pdf)	Safety data sheet - alfentanil	SDS - alfentanil.pdf
Protocol Attachments	2022-1199_1_0001_SDS - buprenorphine.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - buprenorphine.pdf)	Safety data sheet - buprenorphine	SDS - buprenorphine.pdf
Protocol Attachments	2022-1199_1_0001_SDS - diazepam.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - diazepam.pdf)	Safety data sheet - diazepam	SDS - diazepam.pdf
Protocol Attachments	2022-1199_1_0001_SDS - ethanol.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - ethanol.pdf)	Safety data sheet - ethanol	SDS - ethanol.pdf
Protocol Attachments	2022-1199_1_0001_SDS - fentanyl.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - fentanyl.pdf)	Safety data sheet - fentanyl	SDS - fentanyl.pdf
Protocol Attachments	2022-1199_1_0001_SDS - GHB.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - GHB.pdf)	Safety data sheet - GHB	SDS - GHB.pdf
Protocol Attachments	2022-1199_1_0001_SDS - morphine.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - morphine.pdf)	Safety data sheet - morphine	SDS - morphine.pdf
Protocol Attachments	2022-1199_1_0001_SDS - naltrexone.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - naltrexone.pdf)	Safety data sheet - naltrexone	SDS - naltrexone.pdf
Protocol Attachments	2022-1199_1_0001_SDS - oxycodone.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - oxycodone.pdf)	Safety data sheet - oxycodone	SDS - oxycodone.pdf
Protocol Attachments	2022-1199_1_0001_SDS - triazolam.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - triazolam.pdf)	Safety data sheet - triazolam	SDS - triazolam.pdf
Protocol Attachments	2022-1199_1_0001_SDS - zolpdem.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - zolpdem.pdf)	Safety data sheet - zolpidem	SDS - zolpdem.pdf
Research Endpoints/Chair Approval	2022-1199_1_0001_[redacted] IACUC transmittal form - signed.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] IACUC transmittal form - signed.pdf)	signed transmittal form	[redacted] IACUC transmittal form - signed.pdf
Protocol Attachments	2022-1199_1_0001_SDS - flumazenil.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_SDS - flumazenil.pdf)	Safety data sheet - flumazenil	SDS - flumazenil.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - alfentanil.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - alfentanil.pdf)	Hazard form - alfentanil	[redacted] hazardous use form - alfentanil.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - buprenorphine.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - buprenorphine.pdf)	Hazard form - buprenorphine	[redacted] hazardous use form - buprenorphine.pdf

Page	File Name	Description	Original File Name
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - diazepam.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - diazepam.pdf)	Hazard form - diazepam	[redacted] hazardous use form - diazepam.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - ethanol.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - ethanol.pdf)	Hazard form - ethanol	[redacted] hazardous use form - ethanol.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - fentanyl.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - fentanyl.pdf)	Hazard form - fentanyl	[redacted] hazardous use form - fentanyl.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - flumazenil.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - flumazenil.pdf)	Hazard form - flumazenil	[redacted] hazardous use form - flumazenil.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - GHB.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - GHB.pdf)	Hazard form - GHB	[redacted] hazardous use form - GHB.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - midazolam.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - midazolam.pdf)	Hazard form - midazolam	[redacted] hazardous use form - midazolam.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - rhesus_ monkey.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - rhesus monkey.pdf)	Hazard form - rhesus monkey	[redacted] hazardous use form - rhesus monkey.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - morphine.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - morphine.pdf)	Hazard form - morphine	[redacted] hazardous use form - morphine.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - naltrexone.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - naltrexone.pdf)	Hazard form - naltrexone	[redacted] hazardous use form - naltrexone.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - oxycodone.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - oxycodone.pdf)	Hazard form - oxycodone	[redacted] hazardous use form - oxycodone.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - triazolam.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - triazolam.pdf)	Hazard form - triazolam	[redacted] hazardous use form - triazolam.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] hazardous use form - zolpidem.pdf (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] hazardous use form - zolpidem.pdf)	Hazard form - zolpidem	[redacted] hazardous use form - zolpidem.pdf
Protocol Attachments	2022-1199_1_0001_[redacted] - additional experimental agents.docx (https://umc.esirius.cayuse.com/eSirius3g/attachment/2022-1199_1_0001_[redacted] - additional experimental agents.docx)	detailed list of additional experimental agents	[redacted] - additional experimental agents.docx

Amendment Reason

Protocol Number

2022-1199

Protocol Year

3

Protocol Title

Non-human primate models of alcohol abuse: Behavioral pharmacology studies

Approve Date

07/11/2022

Expiration Date

07/11/2025

Full Name

[redacted]

Reason for Change

add new personnel - [redacted] and [redacted]
**note, their CITI certificates and IACUC training forms have been submitted to the IACUC office already

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.

Progress has come in three main areas:

1) GABAergic mechanisms involved in the abuse-related effects of alcohol: We continue to study the role of different GABA-A receptor mechanisms in the reinforcing effects of ethanol in nonhuman primates. Selective ligands are studied in both ethanol and sucrose self-administering monkeys, with sucrose drinkers serving as control subjects. We completed planned studies with ligands selective for alpha2/3 subunit-selective GABA-A receptors (cf. [redacted] et al. 2019). Our current studies are taking a similar approach to investigate the role of alpha4/6 subunit-selective GABA-A receptors. These studies capitalize on a long-standing collaboration with medicinal chemist [redacted] who provides us with novel and selective ligands. Ligands with promising profiles are then evaluated in different relapse models (e.g., [redacted] et al. 2019)

2) Development of a nonhuman primate resurgence model of contingency management therapy: Contingency management therapy (CMT) is one of the most effective treatment strategies for alcohol use disorder. However, significant numbers of individuals still relapse when therapy is terminated. We developed a resurgence model of CMT and have been evaluating adjunctive pharmacotherapies to improve outcomes (e.g., [redacted] et al. 2022).

3) Genotype-dependent opioid-ethanol interactions: Opioids are frequently abused with ethanol. A common variant of the mu opioid receptor gene has been shown to influence responses to both opioids and ethanol, independently. Our studies make use of a population of rhesus monkeys that have been genotyped for a SNP in the monkey mu opioid receptor gene that produces similar physiological and phenotypic outcomes. We show that genotype appears to dictate the nature of the ethanol-opioid interaction. In one genotype, opioids enhance ethanol self-administration; in the other genotype, opioids only suppress ethanol self-administration.

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

[redacted] (2019) $GABA_A$ receptor subtypes and the abuse-related effects of ethanol in rhesus monkeys: Experiments with selective positive allosteric modulators. *Alcohol Clin Exp Ther* 43:791-802 PMCID: 6601614

[redacted] (2019) Enhancement of a cue-exposure therapeutic approach with an alpha5GABA-A inverse agonist in rhesus monkeys. Proceedings from the 81st Annual Meeting of the College on Problems of Drug Dependence, San Antonio, TX

[redacted] (2019) OPRM1 genotype-dependent opioid-ethanol interactions in male rhesus monkeys. *Soc Neurosci Abstr*: XX. Society for Neuroscience 49th Annual Meeting, Chicago, IL

[redacted] (2022) Resurgence of alcohol-maintained behavior in rhesus monkeys: Effects of naltrexone. *Alcohol Clin Exp Res* XX. Research Society on Alcoholism 45th Annual Meeting, Orlando, FL

Invited seminar - 2019: $GABA_A$ receptor subtypes: Potential targets for AUD pharmacotherapies; Wake Forest University

Grant award: R01 AA029023; *$GABA_A$ receptor subtype mechanisms and the abuse-related effects of alcohol*; August 2020 – July 2025

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

I. Animals

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

If yes to 1 -4, answer #5.

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

If the protocol involves breeding: N/A

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:

In April 2022, a graduate student was participating in cage change in [redacted] and had her hand smashed between two clean cages. No contributing factors, just bad luck!

3. What treatment measures were taken:

She scrubbed the wounded area with a betadine scrub for 15 min, then proceeded to Student/Employee Health. She ultimately received 3 stitches to close her wound.

Cayuse Experimental Agent Instructions

For agents not in the "Available Agents" table, complete the table below and attached to your submission.

If none of your experimental agents are listed in the table, select "Other", complete the table below and attached to your submission.

Provide the following information:

Agent	Dose	Volume	Vehicle	Route	Frequency	NDC or CAS#	Hazard?	Pharmaceutical Grade
Gamma hydroxybutyric acid (GHB)	30-300 mg/kg	0.01 – 0.1 ml/kg	Sterile water	IM	1-10 days/test	502-85-2	Yes	☐Yes ☒No
d-cycloserine	1-10 mg/kg	0.01 – 0.1 ml/kg	Sterile water	IM	Daily in specific experimental phases	68-41-7	No	☐Yes ☒No
Bretazenil (experimental GABA/BZ agonist)	0.03 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/ethanol/propylene glycol	IM	1-10 days/test	84379-13-5	No	☐Yes ☒No
Cook #6 (experimental GABA/BZ agonist)	0.3 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
Diazepam	0.1 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	In case of ethanol withdrawal	439-14-5	Yes	☐Yes ☒No
DK-I-56-I (experimental GABA/BZ agonist)	0.3 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
Gaboxadol (experimental GABA/BZ agonist)	0.03 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	85118-33-8	No	☐Yes ☒No
HZ-166 (experimental GABA/BZ agonist)	3 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
L-838417 (experimental GABA/BZ agonist)	0.3 – mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	286456-42-6	No	☐Yes ☒No
Midazolam	IM: 0.01 – 10 mg/kg	IM: 0.01 – 0.1 ml/kg	IM: saline/sterile water	IM/oral	IM: 1-10 days/test	59467-96-8	Yes	☒Yes ☐No

Ro15-4513 (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
RY-23 (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
TB21007 (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
3-PBC (experimental GABA/BZ antagonist)	1 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
B-CCt (experimental GABA/BZ antagonist)	0.3 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
DM-D-053 (experimental GABA/BZ antagonist)	0.1 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
Flumazenil	0.01 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	78755-81-4	Yes	☐Yes ☒No
Xli-093 (experimental GABA/BZ antagonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
Alfentanil	0.003 – 0.3 mg/kg	0.01 – 0.1 ml/kg	Sterile water	IM	1-10 days/test	71195-58-9	Yes	☐Yes ☒No
Buprenorphine HCl	0.01 – 1 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water	IM	1-10 days/test	53152-21-9	Yes	☐Yes ☒No
Fentanyl citrate	0.003 – 0.3 mg/kg	0.01 – 0.1 ml/kg	Sterile water	IM	1-10 days/test	990-73-8	Yes	☐Yes ☒No
Morphine	0.1 – 100 mg/kg	0.01 – 0.1 ml/kg	Sterile water	IM	1-10 days/test	6211-15-0	Yes	☐Yes ☒No
Oxycodone	IM: 0.1 – 10 mg/kg Oral: set by monkey	IM: 0.01 – 0.1 ml/kg Oral: set by monkey	IM: saline Oral: tap water	IM; oral	IM: 1-10 days/test Oral: Daily	76-42-6	Yes	☐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, Environmental Health and Safety. Choose the "MSDS On-Line" link under "Helpful LInks".

	Oral: set by the monkey	Oral: set by the monkey	Oral: tap water		Oral: daily			
MP-III-080 (experimental GABA/BZ agonist)	0.1 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
PWZ-029 (experimental GABA/BZ agonist)	0.3 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
QH-II-066 (experimental GABA/BZ agonist)	0.1 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
TP003 (experimental GABA/BZ agonist)	0.01 – 30 mg/kg	0.01 – 0.1 ml/kg	saline/sterile water/propylene glycol	IM	1-10 days/test	628690-75-5	No	☐Yes ☒No
TPA-023B (experimental GABA/BZ agonist)	IM: 0.1 – 1 mg/kg Oral: set by the monkey	IM: 0.01 – 0.1 ml/kg Oral: set by the monkey	IM: saline/sterile water/propylene glycol Oral: propylene glycol/tap water	IM/oral	IM: 1-10 days/test Oral: daily	425377-76-0	No	☐Yes ☒No
Triazolam	0.01 – 1 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	28911-01-5	Yes	☐Yes ☒No
XHe-III-74 (experimental GABA/BZ agonist)	0.3 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
YT-III-31 (experimental GABA/BZ agonist)	0.1 – 10 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
Zolpidem	0.1 – 30 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	82626-48-0	Yes	☐Yes ☒No
BCCE (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
L-655,708 (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No
MRK-016 (experimental GABA/BZ inverse agonist)	0.03 – 3 mg/kg	0.01 – 0.1 ml/kg	Saline/sterile water/propylene glycol	IM	1-10 days/test	N/A	No	☐Yes ☒No

The University of Mississippi Medical Center

Animal Activity Protocol
Transmittal Form

IACUC - Institutional Animal Care and Use Committee
Telephone 601 815-5006 / Facsimile 601 815-5010
iacuc@umc.edu

PI: [redacted]

Project Title: Non-human primate models of alcohol abuse: Behavioral pharmacology studies

Funding Source: NIH/NIAAA R01AA029023

When does the Animal Activity Protocol need to be reviewed by the IACUC?

☑ At the next scheduled IACUC meeting because funds (departmental or grant) are currently available for the project.

☐ When the corresponding grant application is identified for funding. (PI will notify the IACUC.)

Species: Rhesus monkeys (Macaca mulatta)

This project contains:

☑	Appendix A	Environmental Enhancement/ Enrichment (required for NHP)	☐	Appendix H	Multiple Survival Surgeries
☐	Appendix B	Breeding Programs	☐	Appendix I	Food and/or Fluid Restriction
☐	Appendix C	Surgery & Management of Surgical Pain	☐	Appendix J	Animal Pain and/or Distress
☑	Appendix D	Collection of Biological Samples	☑	Appendix K	Progress Report (required for FSR submissions)
☐	Appendix E	Antibody Production	☑	Appendix L	Behavior Testing and Training
☑	Appendix F	Administration of Drugs/Test Compounds	☐	Appendix N	Use of Expired Medical Material or Devices
☐	Appendix G	Prolonged Physical Restraint	☑	Hazard Use	Biological, Chemical, and/or Radiation

Review by Department Chair, Head, Director, or Dean (If PI is Dept. Chair/Head/Director, the Dean must sign)

The signature below certifies acknowledgement that this research is in keeping with the standards set by your department/unit, all UMMC policies and that facility, equipment, funds, and personnel are appropriately committed to this project.

Name: [redacted]

Signature: [redacted]

Title: Interim Chair

Chair, Head, Director, or Dean

Date: 05-24-2022

Carefully review the application to ensure it is complete, contains sufficiently detailed responses to all questions and all attachments. <u>Incomplete applications will be returned without IACUC review or approval,</u> potentially delaying the research. Contact the Office of Animal Welfare for questions or assistance.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Alfentanil	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Alfentanil is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted].

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Buprenorphine HCl	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☑ Reproductive hazard	☐ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	Lab prep of material	Animal exposure in CCR	Specify type
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	Lab prep of material	Animal exposure in CCR
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Buprenorphine is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Diazepam	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Diazepam is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Ethanol	☐	☑	☐

Potential routes of exposure	☐ Inhalation	☑ Skin Contact/ Skin Absorption	☑ Ingestion	☐ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	Lab prep of material	Animal exposure in CCR	Specify type
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	Lab prep of material	Animal exposure in CCR
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in a flammable cabinet	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Ethanol is not a scheduled compound; however, it is flammable and, thus, will be stored appropriately in a flammable cabinet.

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Fentanyl citrate	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Fentanyl is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent using this protocol. For assistance: Biological, ext. [redacted]; Chemical, ext. [redacted]; Radiation, ext. [redacted]; After hours, ext. [redacted]

MSDS attached ☑
PI: [redacted]
Contact Number: [redacted]

Hazard Use Area: Lab room # [redacted]
CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
flumazenil	☐ABSL 1 ☐ABSL 2	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☐ Toxic ☑ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Lab Coat/Gown	☑	☑	standard lab coat
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):		

Solubility property of chemical	☐ Water soluble; ☑ Non water soluble; ☐ Special

☐Yes	☑No	Is the proposed exposure dosages/amounts considered determental to humans?
☐Yes	☑No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

*See back for additional comments

☐Additional Comments:

We do not anticipate any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and lab coats) as indicated in the material safety data sheets to maximize protection from exposure risk.

The levels of drug administered to or by the monkeys coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent using this protocol. For assistance: Biological, ext. [redacted]; Chemical, ext. [redacted]; Radiation, ext. [redacted]; After hours, ext. [redacted]

MSDS attached ☑
PI: [redacted]
Contact Number: [redacted]

Hazard Use Area: Lab room # [redacted]
CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
GHB	☐ABSL 1 ☐ABSL 2	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Lab Coat/Gown	☑	☑	standard lab coat
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

Solubility property of chemical	☑ Water soluble; ☐ Non water soluble; ☐ Special

☐Yes	☑No	Is the proposed exposure dosages/amounts considered determental to humans?
☐Yes	☑No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

*See back for additional comments

☐Additional Comments:

We do not anticipate any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and lab coats) as indicated in the material safety data sheets to maximize protection from exposure risk. Gamma hydroxybutyric acid (i.e., GHB) is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Midazolam HCl	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☑ Reproductive hazard	☐ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard Nitrile
Mask	☑	☑	standard Facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	Stored in double-locked safe.	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection, using an enclosed scale) as indicated in the material safety data sheets to maximize protection from exposure risk. Midazolam is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Morphine sulfate	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	Lab prep of material	Animal exposure in CCR	Specify type
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	Lab prep of material	Animal exposure in CCR
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Morphine is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted].

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Naltrexone HCl	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):		

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Naltrexone is not a scheduled compound and, thus, will be stored appropriately in the laboratory drug room ([redacted]).

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent using this protocol. For assistance: Biological, ext. [redacted]
Chemical, ext. [redacted]; Radiation, ext. [redacted]; After hours, ext. [redacted].

MSDS attached ☐
PI: [redacted]
Contact Number: [redacted]

Hazard Use Area: Lab room # N/A

CCR Room # NHP dedicated CCR rooms

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Herpes B potential carrier	☐ABSL 1 ☑ABSL 2	☐	☐

Potential routes of exposure	☐ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☐ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☐	☑	standard
Mask	☐	☑	standard
Eye Protection	☐	☑	standard
Lead Protection/Dosimetry	☐	☐	
Lab Coat/Gown	☐	☑	standard
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):		

Solubility property of chemical	☐ Water soluble; ☐ Non water soluble; ☐ Special

☑Yes	☐No	Is the proposed exposure dosages/amounts considered determental to humans?
☑Yes	☐No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

*See back for additional comments

☐Additional Comments:

Rhesus macaques are potential carriers of Macacine herpesvirus 1. ABSL-2 practices are followed when working with NHPs. We have an approved IBC protocol for our work with this species.

PPE: gloves, mask, eye protection, and long sleeves

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted].

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Oxycodone	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☑ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Oxycodone is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. [redacted]

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Triazolam	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☐ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	**Lab prep of material**	**Animal exposure in CCR**	**Specify type**
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	**Lab prep of material**	**Animal exposure in CCR**
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Nor does the Safety Data Sheet indicate that triazolam is a health hazard. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Triazolam is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.

The University of Mississippi Medical Center

Hazardous Agent Use in Animal Studies

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

Complete a form for each hazardous agent useing this protocol. For assistance contact Office of Environmental Health and Safety, ext. 4-1988.

☑ MSDS attached

Hazard Use Area: Lab room # [redacted]

PI: [redacted]

CCR Room # [redacted]

	Category		
Agent Used	Biological	Chemical	Radioisotope/Ionizing
Zolpidem	☐	☑	☐

Potential routes of exposure	☑ Inhalation	☑ Skin Contact/ Skin Absorption	☐ Ingestion	☑ Injection
	☑ Mucosal/Ocular	☐ External Radiation		
Other:				
Potential hazard to personnel	☐ Reproductive hazard	☐ Toxic ☐ Corrosive	☐ Carcinogen ☐ Respiratory	☐ Agent causes disease
Other:				

PPE Required:	Lab prep of material	Animal exposure in CCR	Specify type
Gloves	☑	☑	standard nitrile
Mask	☑	☑	standard facemask
Eye Protection	☑	☑	standard face shield
Lead Protection/Dosimetry	☐	☐	
Other			

Room Safety Features required:	Lab prep of material	Animal exposure in CCR
Biological Safety Cabinet	☐	☐
Chemical Fume Hood	☐	☐
Other (describe):	stored in double locked safe	

☐ Yes	☑ No	Is the proposed exposure dosages/amounts considered determental to humans?
☐ Yes	☑ No	Are hazardous agents excreted or shed into animal bedding or exhaled?

Waste Handling:	☑ Carcass and bedding- Standard biological waste disposal
	☐ Autoclave cages
	☐ Chemical treatment:
	Other:

Reviewed by Biological Safety Officer Date

Reviewed by Chemical Safety Officer Date

*See back for additional comments

Additional comments:

We do not anticipate nor have we experienced any harmful exposure events in the drug preparation and experimental administration process. Nor does the Safety Data Sheet indicate any potential health hazard. Drugs are prepared in concentrations of sufficient dilution to be non-toxic in the event of an accidental external exposure. However, preparation and administration of drugs are conducted with good laboratory practices (i.e., with gloves, masks, and eye protection) as indicated in the material safety data sheets to maximize protection from exposure risk. Zolpidem is a DEA scheduled compound. Accordingly, it will be stored in a double-locked safe under the DEA license of [redacted]

The levels of drug administered to or by the monkeys, coupled with the disposition of the drug within the animal, will not result in accumulation of a hazardous amount of compound within the animal or on the surrounding surfaces, including the bedding. Thus, standard bedding disposal procedures are warranted.