UNIVERSITY OF MINNESOTA

Research Subjects' Protection Programs
Institutional Review Board: Human Subjects Committee (IRB)
Institutional Animal Care and Use Committee (IACUC)

Mayo Mail Code 820
D-528 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455

612-626-5654
Fax: 612-626-6061
irb@umn.edu
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November 12, 2004

Marilyn E. Carroll
Psychiatry
MMC 392 Mayo
420 Delaware
Minneapolis MN 55455

Re: "Imaging the Effects of Drug Use and Cessation on Monkey Brain"
"Primate Model of Drug Abuse: Intervention Strategies"
Animal Subjects Code Number: **0410A64755**

Dear Dr. Carroll:

At its meeting on November 9, 2004 the Institutional Animal Care and Use Committee (IACUC) reviewed the referenced application. The following stipulations must be satisfied before approval can be granted. You must receive IACUC approval before animals may be ordered or the study initiated. The Committee made the following stipulations:

1) You state in response to question 3b that the goals are the same as those listed in the original protocol. While it appears your response to question 3a details those goals, please provide a response to question 3b that details what was accomplished during the prior approval period.
2) The number of animals requested and detailed is inconsistent throughout your submitted protocol. You state on your animal request table that you need to purchase 38 NHPs, you detail the use of 37 animals on page 10, and then in your justification section you state that a total of 34 animals will be used in the proposed experiments. One of the reasons that IACUC requests that investigators complete an IACUC application distinct from their grant, is so that the responses to the animal request table, 3c, 3d, and 4c concisely correlate using the same terminology. It appears that you have cut and pasted text from your grant, and your responses do not match. Please clarify the number of animals requested for purchase, transfer, and then clarify the justification for that number.
3) The title of this protocol mentions imaging the effects of drug use and cessation, but none of the procedures seem to be associated with that work. Please clarify this discrepancy.
4) You have listed animals on this study as pain class A animals. The committee is concerned that animals may be considered pain class B if imaging is conducted or if animals experience withdrawal symptoms that they may be considered pain class C. Please justify your pain classification by addressing the committee's concerns.
5) It is unclear if animals on this protocol develop a tolerance to agents administered, or if any animals develop a dependence on agents administered. Please provide any specific citations and brief summary that addresses these concerns.

6) Please provide specific scientific justification for why animals are placed on food restriction.
7) Please clarify the endpoints of this study. It is understood that animals may be used on multiple experiments, but it is unclear how long animals undergo each procedure and then how long of a washout period exists between experiments. Please address this concern by explaining the length of experiments and length of washout periods.
8) Please note that the committee can only approve the use of agents detailed in this protocol, and that if you desire to test additional agents, then you will need to submit a change in protocol request and wait for approval before proceeding.
9) The committee is unclear if any animal work at CMRR is intended to be covered by this protocol. Please address this concern.
10) All employees working with animals are required to attend an orientation/training seminar. To date, *Kacie Griffin and Joey Thome* have not completed this seminar. The seminar is offered twice a month and the schedule is available through the following web site: http://www.iacuc.umn.edu/training/index.cfm. As an alternative to attending the seminar, employees may also review the material entitled Humane Animal Care and Use at the University of Minnesota located on the same web site. Your application will not receive final approval until this process is completed.

Please return your response to these questions to this office as soon as possible. We will process the final approval as quickly as possible at that time. If you have any questions, please call the IACUC office at (612) 626-5654.

Sincerely,

Jeffery Perkey, CIP
Executive Assistant, IACUC

JP/ejv

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Institution For Animal Care And Use Committee
Minutes
Tuesday
November 9, 2004

Meeting Convened at: 12:30 p.m.
Meeting Adjourned at: 3:30 p.m.

Chairperson: Tom Molitor

Members in Attendance:
Brian Crooker (M), Ann Fitzpatrick (O), Cynthia Gillett (A), Malinda Hartman (M), Cathy Marquardt (M), Tom Molitor (M), Melissa Nellis (M), Bruce Overmier (M), Kathy Scoggin (M), Geoffrey Sirc (M), George Wilcox (M)

Members Absent:
Alvin Beitz (M), Marilyn Bennett (A), Marcia Brower (A), Cathy Carlson (A), Angela Craig (M), Carolyn Fairbanks (A), M. Kent Froberg (M), Cory Goracke-Postle (A), Roland Gunther (A), Moira Keane (A), Edward Knych (A), Susan McClanahan (A), Andrew Morgan (A), Sally Noll (A), Andrew Rivard (M), Ava Trent (M), Scott Walden (A)

Quorum Requirement: 8

The following Minutes were reviewed and approved by the chairperson in attendance at the meeting.

Tom Molitor

Tom Molitor, chair

12/1/04

Date

Carroll, Marilyn E. **"Imaging the Effects of Drug Use and Cessation on Monkey Brain" 0410A64755**

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The title of this protocol mentions imaging the effects of drug use and cessation, but none of the procedures seem to be associated with that work. Please clarify this discrepancy.
You have listed animals on this study as pain class A animals. The committee is concerned that animals may be considered pain class B if imaging is conducted or if animals experience withdrawal symptoms that they may be considered pain class C. Please justify your pain classification by addressing the committee's concerns.
It is unclear if animals on this protocol develop a tolerance to agents administered, or if any animals develop a dependence on agents administered. Please provide any specific citations and brief summary that addresses these concerns.
Please provide specific scientific justification for why animals are placed on food restriction.
Please clarify the endpoints of this study. It is understood that animals may be used on multiple experiments, but it is unclear how long animals undergo each procedure and then how long of a washout period exists between experiments. Please address this concern by explaining the length of experiments and length of washout periods.
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All employees working with animals are required to attend an orientation/training seminar. To date, Kacie Griffin and Joey Thome have not completed this seminar. The seminar is offered twice a month and the schedule is available through the following web site: http://www.iacuc.umn.edu/training/index.cfm. As an alternative to attending the seminar, employees may also review the material entitled Humane Animal Care and Use at the University of Minnesota located on the same web site. Your application will not receive final approval until this process is completed.
11/0/0

0F. Checklist for submitting a complete application

This checklist must be included as part of your application. Check all that pertain to your project. Where indicated complete the appropriate appendices and attach as part of your application.

☐ **Euthanasia and harvesting of tissue only**

☒ **Behavioral studies**

☐ **Animals are sent to slaughter or put into the human food chain**

☐ **Alternatives to animals classified in categories B or C - *Appendix A***

☐ **Breeding of animals - *Appendix B*** (see instructions in section 1 to determine if completing a separate breeding protocol is necessary)

☐ **Housing of animals outside of the primary housing area (> 12 hours) - *Appendix D***

☐ **Teaching/Classroom Protocol – *Appendix E***

☐ **Surgery – Please check: ☐ Survival surgery. ☐ Non-survival surgery. - *Appendix F***

☐ **Use of these specific agents in animals - *Appendix G***

- ☐ **Part I: Hazardous Chemicals**
- ☐ **Part II: Radiation**
- ☐ **Part III: Infectious agents and work with human blood and body fluids**
- ☐ **Part IV: Recombinant DNA including transgenic mice**

☒ **Use of Controlled substances – *Appendix C***

Important note regarding the use of non-pharmaceutical grade drugs

Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used after specific review and approval is granted by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical grade compounds in animals. See http://www.aphis.usda.gov/ac/policy/policy3.pdf

☒ **Immunization, antibody or ascites production, or collection of other body fluids - *Appendix H***

☒ **Pharmacologic/toxicologic studies - *Appendix I***

☒ **Dietary manipulations or fluid restriction - *Appendix J***

☐ **Conscious restraint for more than one hour - *Appendix K***

☐ **Free-ranging wildlife - *Appendix L***

☐ **Client-owned animals - *Appendix M* Client Consent Form**

☒ **Receiving external funds for this research - *Appendix O***

☐ **Using Monoclonal Antibody Production, mouse ascites method – *Appendix P***

0G. Source of Funding

Name of funding source:	NIDA-NIH

Grant: ☐ Will be submitted.
☐ Submitted.
☒ Approved. If approved, what is the duration of approval: ☒ 5 years ☐ Other:

This application must be written for a maximum of three years only.

If you are receiving external funds for this research, include an Appendix O for each source of funding.

0H. Personnel working on study

List the personnel that will be working on this study below. Indicated their role on the study, if they will be working with animals, and if they should receive mail about the study from IACUC. Prior to approval being granted, everyone working with animals below must have completed the IACUC animal use certification (http://www.iacuc.umn.edu/training).

Name (Last Name, First Name MI)	U of M Employee or Student ID	U of M x.500 ID (ex. smith001)	Phone number	Role in Project	Works with Animals	Receive Mail from IACUC*
Marilyn Carroll			6-6289	Advisor	☒	☐
Jennifer Perry			6-6301	Lab Staff	☒	☐
Joey Thorne			"	Lab Staff	☒	☐
Lisa Normile			"	Lab Staff	☒	☐
David Batulis			"	Lab Staff	☒	☐
Chris Sigstad			"	Lab Staff	☒	☐
Kerry Landry			"	Lab Staff	☒	☐
Chris Koch			"	Lab Staff	☒	☐
Kacie Griffin			"	Lab Staff	☐	☐
Sarah Nelson			"	Lab Staff	☐	☐
Nick Cmiel			"	Lab Staff	☐	☐
Derek Thermes			"	Lab Staff	☒	☐

**If a person to receive mail [illegible] not a [illegible]ersity employee (not listed with an employee ID or x500 ID), attach a sheet with their mailing address, phone, fax and email information with this application.*

0I. Conflict of Interest

Federal Guidelines emphasize the importance of assuring there are no conflicts of interest in research projects that could affect the welfare of animal subjects. If this study involves or presents a potential conflict of interest, additional information will need to be provided to the IACUC. Examples of potential conflicts of interest may include, but are not limited to:

- A researcher or family member participating in research on a technology, process or product owned by a business in which the faculty member holds a financial interest
- A researcher participating in research on a technology, process or product developed by that researcher
- A researcher or family member assuming an executive position in a business engaged in commercial or research activities related to the researchers University responsibilities
- A researcher or family member serving on the Board of Directors of a business from which that member receives University-supervised Sponsored Research Support
- A researcher receiving $10,000 or more in consulting income from a business that funds his or her research

University of Minnesota Researchers, please refer to:
http://www1.umn.edu/regents/policies/academic/ConflictofInterest.html

Fairview Health System Researchers, please refer to:
http://www.fairview.org/prof/research

Do any of the Investigators or personnel listed on this research have a potential conflict of interest associated with this study?

☒ No. Skip to section 1.
☐ Yes.

If yes, identify the individual(s):

Has this potential conflict of interest been disclosed and managed?

☐ No.
If you are a University of Minnesota researcher, please disclose your potential conflict of interest online for review by your Department Head and Dean via the Report of External Professional Activities (REPA) at https://egms.umn.edu/REPA/

☐ Yes.
The IACUC will verify that a management plan is in place with the Conflict Management Committee (CMC). If the CMC does not have an approved management plan for this research, the CMC will contact the individual(s) listed for additional information.

Final IACUC approval cannot be granted until all potential conflict matters are settled. The IACUC requires a recommendation from the CMC regarding disclosure to subjects and management of the conflict.

1. Animal Request by Species, Pain Class, and Source of Animals

List the animals requested for use, including the pain class for each, and the number that will be used over the three-year period in the column corresponding to the source of animals.

Animal Request Table

Species	Pain Class[1] (one per row)	Number of Animals to be Used over Three-year Period by Source					Total Number
		# Purchased (Or received from other institution)	# Transferred[2]		# Bred In-house[3]	# Other (Specify: captured wildlife, observation)	
			From IACUC study number	# of Animals			
Rhesus monkey	A	38					38

If more than one species is listed, complete Part B of this form for each species.

[1]**Pain Classes**

Class A: No pain, distress or use of pain-relieving drugs: Examples include post-mortem tissue harvest; and routine procedures causing only transitory discomfort such as venipuncture, injections, ear tagging, use of non-inflammatory adjuvants, etc.

Class B: Pain/distress WITH appropriate analgesia/anesthesia/tranquilizers. Procedures involving accompanying pain or distress to the animals and for which the appropriate anesthetic (for surgery), analgesic (for inflammation or pain), or tranquilizing drug are used. **You must complete Appendix A.**

Class C: Pain/distress WITHOUT analgesia/anesthesia/ tranquilizers. Procedures involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesics or tranquilizing drugs would adversely affect the procedures, results or interpretation. **You must complete Appendix A.**

[2]**Number Transferred**

Also include the IACUC Study Code Number from which the animals will be transferred (ex. 0202A12345 | 50). This form does not transfer the animals to this protocol once it is approved. The Protocol Transfer Request Form (http://www.ahc.umn.edu/rar/transfer.pdf) *must* be completed and submitted to RAR.

[3]**Notes on breeding & transferring:**

If you intend to breed animals for use by multiple researchers or multiple protocols, complete the *Breeding Protocol Form* to cover the breeding and continue completing this from to cover the experimental use of the animals.

If you will be transferring animals from a different IACUC protocol (including breeding protocols) to this experimental protocol include these animals in the "Transferred" column, not the "Produced by in-house breeding" column, along with the existing IACUC Study Code Number (ex. 0202A12345 | 50). The numbers proposed and justified in this application should only apply to the experimental animals.

If you will complete and submit a breeding protocol along with this application, fill in the number of animals that will be transferred from that breeding protocol to this IACUC study in the "Transferred" column and leave the "From IACUC study code #" column blank.

If you intend to breed animals for use in only this protocol, complete only this form listing the total number of bred animals in the "Produced by in-house breeding" column and include Appendix B.

Please call the RSPP Office at 612-626-5654 if you have any questions about how to complete either form.

2. Housing

2A. Will animals be housed in Research Animal Resources (RAR) – managed facilities?

☒ Yes.
☐ No.

If no, list the facility (building and room #) where animals are or will be housed: (such as Private lab in Mayo, Veterinary Teaching Hospital, School of Medicine Duluth, Experimental Stations – specify, or other – specify)

Building	Room number	Campus

2B. Will animals be held outside of a centrally managed housing area for more than 12 hours?

(such as Jackson, Animal Science, Experimental Stations – specify, or other – specify)

☐ Yes. *Include Appendix D and fill in the table below.*
☒ No.

Building	Room number	Campus

2C. If live animals will be removed from the above areas, please indicate the procedures to be performed, the location and name of contact person.

Procedure(s) to be performed:	
Building:	Room:
Name of contact:	Phone number:

2D. Will animals be transported through public places by anyone other than RAR?

☐ Yes.
☒ No.

If yes, complete the following:

From where to where will animals be transported?	
Via what route will the animals be transported?	
Who will transport the animals?	
What equipment will be used to transport them?	
At what time(s) will transport occur?	
Contact RAR for appropriate transportation procedures at 612-624-6169	

3. Specific Aims & Details of Animal Use

3A. What is the goal/specific aim of this project? What is the research or development question?
Describe the relevance of the study to advancing scientific knowledge and/or the benefits of the study to human and/or animal health. Provide sufficient information to indicate that the potential new knowledge from the project justifies the use of animals. Jargon should be avoided or explicitly explained (please define all acronyms).

Goals of the proposed research are to use a rhesus monkey model of drug abuse to study factors affecting vulnerability to drug abuse and to evaluate behavioral and pharmacological treatment interventions. Routes of administration that have been developed in this laboratory will include oral drug self-administration and smoking. When using the oral route of self-administration, liquid deliveries are contingent upon lip-contact responses. Smoke deliveries are contingent upon inhalation responses. Vulnerability factors to be examined are sex and phase of the menstrual cycle as well as patterns/duration of access to drugs. Initial work with rats indicates that escalation from drug use to abuse is dependent upon sex, hormonal status, and the amount and duration of access. The proposed work will extend these findings to monkeys, other drugs, and measures of reinforcing efficacy. In addition, the question of whether differential access to one drug affects acquisition of self-administration of a second drug will be examined. Well-accepted measures of the reinforcing efficacy to drugs, behavioral economic demand curve analysis and progressive ratio (PR) schedules will be used to determine how these predisposing factors ultimately affect the reinforcing potential of selected drugs. The drugs that will be studied are cocaine, ethanol, heroin, methadone and phencyclidine (PCP. Behavior maintained by food and/or liquid saccharin will be used as a control for drug-selective effects. The behavioral economic measures will also be used to quantify the extent to which these drug and nondrug substances substitute for each other. These studies will inform us about the effectiveness of substituting nondrug items for drugs in treatment, as well for predicting polydrug abuse by now well one form of drug abuse substitutes for another. The use of nondrug reinforcers as a behavioral treatment will also be compared in male and female monkeys and during 3 phases of the menstrual cycle. Potential treatment medications will also be examined in male and female monkeys using a behavioral economic approach. Two different types of drugs that have produced promising preliminary results are proposed: bremazocine, an agonist at the kappa opioid receptor, and baclofen, a $GABA_B$ agonist. Finally, the behavioral (alternative reinforcer) and pharmacological treatments will be combined and compared to the effects of each given alone in both males and females. The results of the proposed 10 experiments will provide valuable information about major vulnerability factors and several behavioral and pharmacological treatment approaches for drug abuse. It is hoped that this information will lead to earlier and more effective prevention and treatment of drug abuse.

The proposed experiments are designed to evaluate factors that affect vulnerability to drug abuse such as sex, phase of the menstrual cycle; and patterns/duration of drug exposure using rhesus monkeys that are trained to self-administer drugs by the oral route (drinking and smoking). Experiments are also designed to examine behavioral and pharmacological interventions for drug abuse. The following are the specific aims.

1. Vulnerability/Sex and Hormonal Status To examine differences in sex and hormonal

status with respect to the reinforcing effects of drugs and other substances. Behavioral economic measures and progressive-ratio (PR) schedules will be used to evaluate relative reinforcing efficacy of drugs under different conditions. Behavior maintained by orally self-administered phencyclidine (PCP), smoked cocaine base, heroin, food, and saccharin will be compared across males and females and during the phases of the menstrual cycle. Self-administration of these substances will also be compared in male and female monkeys when behavioral (alternative reinforcers) and pharmacological (e.g., baclofen, bremazocine) treatments are administered. It is hypothesized that the drugs will have greater reinforcing efficacy in females than in males, and females will show greater reductions in intake under the treatment conditions. It is also expected that drug self-administration will increase in females during the late follicular phase of the menstrual cycle when estrogen levels are high and progesterone is low.

2. Vulnerability/Access Conditions To investigate the transition from stable drug use to escalating levels of drug addiction by comparing 3 groups of male and female monkeys exposed to drugs (e.g., PCP) for either short (1 hr) or long (6 and 12 hr) periods each day. It is hypothesized that escalation will be greater in females and with longer exposure durations. The reinforcing efficacy of PCP will be examined using a PR schedule and behavioral economic analysis of demand. The groups will also be compared with respect to their rate and success of acquisition of self-administration of a second drug (e.g., methadone) as a function of their exposure history.

3. Treatment Strategies/Behavioral Interventions To use behavioral economic measures to quantify the extent to which cocaine, ethanol, heroin, PCP and saccharin substitute for each other when the price or fixed ratio (FR) schedule value of one substance is increased and the other (potential substitute) remains fixed. This model will initially be developed in male monkeys to evaluate and quantify the optimal conditions under which alternative nondrug substances serve as substitutes for drugs and for predicting polydrug abuse by how well one drug substitutes for another. It is predicted that drugs will be better substitutes than nondrug substances, and substitution of one substance for another may be asymmetrical.

4. Treatment Strategies/Pharmacological Interventions To determine the effect of pretreatment with baclofen, a $GABA_B$ agonist, and bremazocine, a kappa opioid agonist, on behavior maintained by cocaine, heroin, PCP, saccharin and food in males vs. females. Behavioral economic measures will be used to examine the effect of different doses of these medications on the demand for self-administered drugs and other substances. It is hypothesized that the drugs will produce dose-dependent decreases in drug and saccharin intake, but they will have less of an effect on food intake. It is predicted that females will show greater treatment effects.

5. Treatment Strategies/Combined Behavioral and Pharmacological Interventions To use the most effective and selective (for drug-reinforced behavior) doses of the treatment drugs listed in Aim 4 in combination with the alternative reinforcer treatments (Aim 3) to determine whether the combined treatment is more successful in reducing the demand for

drug than either treatment alone. Male and female monkeys will be compared, and it is hypothesized that the combined behavioral and pharmacological treatments will result in a greater suppression of drug self-administration than either treatment alone.

3B. If this application is a continuation of an ongoing project, please state concisely how these goals differ from those in the original application and what was accomplished during the prior approval period.

Same as original goals

3C. Provide a complete and accurate description of what procedures will be performed on/with the animals. Answer in lay language or language understood by a person unfamiliar with your area of research (*define all acronyms*). Jargon should be avoided or explicitly explained. *Do not cut and paste from a grant proposal or include language or explanations that are not relevant to animal use.*

Provide sufficient detail to allow evaluation by the IACUC. You are strongly encouraged to use a diagram or chart to explain complex designs. **(Use additional pages if needed)**

- Describe all procedures, their frequency and time points over the course of the experiments. Be certain to detail the pain classification of each animal group. This should correspond to the information you provided in the **Animal Request Table** (Section 1).
- Include how long the animals will be maintained. Include dose, route of administration and frequency of any drugs to be administered.
- Describe methods used in behavior studies (including use of noxious stimuli or other methods of positive or negative reinforcement).
- Surgery should be described here only as it relates to the study design. Surgical details should be provided in Appendix F.
- For animals used in agricultural projects, you may reference the study code number of the IACUC approved Standard Operating Procedures for the housing facility and husbandry, as applicable.

See attached

3D. For each species listed on the "Animal Request Table" in section 1, list your experimental and control groups. Indicate the number of animals in each and to which pain classification (A, B or C) they belong (a table format is highly recommended). The number of animals must add up to the total number of animals requested in section 1 and, if applicable, those discussed in Appendix B (breeding). This response should correspond to the response in question 3C.

A - 38 rhesus monkeys

D. Experimental Design and Methods

1. General Methods

Animals. Subjects will be 3 rhesus monkeys, 4 to 12 years old, 14 females and 20 males. Nine new monkeys will be added to the colony to replace older animals. Under most experimental conditions the monkeys will be maintained at 85% of their free-feeding body weights by allowing them unlimited access to food until there is no increase in body weight for 3 successive biweekly measurements. Weights will be reduced by feeding *slightly smaller* amounts (150-200 g/day) *than would be consumed ad libitum*. When the target weights are reached, the daily food allotment will be adjusted to maintain them. The 85% weights usually range from 9-11 kg in males and 5-7 kg in females. The monkeys are housed in their experimental chambers in temperature- (24°C) and humidity-controlled rooms that hold 8-14 monkeys. Every 6 months each monkey is anesthetized with an i.m. injection of Ketalar® while a TB test is given. At this time the veterinary technician also clips the monkeys' nails, cleans their teeth and gives them a general physical examination.

The 34 monkeys will be divided into 3 groups: Group 1 = 7 males, 7 females; Group 2 = 7 males, 7 females; Group 3 = 6 males. Each group will participate in 3-4 experiments as shown in Table 9. *Some of the monkeys used in the proposed research will be drug-experienced at the start of the experiments, and others will be experimentally and drug-naive. Data will be compared across these two groups to determine whether pharmacological and/or behavioral history is a factor that affects the results. However, 23 years of previous experience with rhesus monkeys has shown that behavioral and pharmacological history may influence the rate or success of acquisition of drug self-administration (Campbell and Carroll, 2000a; Campbell et al., 1998; Carroll, 1984; Carroll et al., 1984), but maintenance levels of drug self-administration are relatively unaffected on a long-term basis (Campbell and Carroll, 2000).*

Apparatus. Each animal lives in a custom-made stainless steel test chamber (Lab Products, Inc.) that also serves as an operant chamber. The chamber consists of solid side and back walls, a barred front wall/door, and a plastic coated metal grid floor. An operant panel is attached to the outside of one of the side walls. The panel contains 2 rass drinking spouts (or 1 drinking spout and 1 smoking spout), a centrally-located lever, and 3 stimulus lights located above these components. The stimulus lights above the spouts and lever are illuminated red to signal food or that a smoking trial has begun and green to signal liquid or smoke availability. A flashing green light indicates drug and a solid-on green light signals water. There is a pellet receptacle below the lever, connected by a chute to a universal feeder (Gerbrands) mounted on the top of the cage. Holes punched in the side walls allow response and delivery devices and lights to protrude through the cage walls.

Drinking Device. The drinking devices, which are 2.7 cm long and 0.7 cm in diameter, are mounted directly behind a Plexiglas plate that contains 4 green and white stimulus lights. Two white lights are illuminated for the duration of each lip contact when water is available, and 2 green lights are illuminated when drug or saccharin is available. The response is a lip-contact on the brass spout. The lights behind the clear plate that holds the spout remain on for the duration of the lip contact. After a specified number of lip contacts, under an FR schedule, a solenoid valve is opened for an amount of time necessary (e.g., 120 msec) to deliver a fixed volume of liquid (0.6 ml). Liquid flows through the spout until this amount is delivered or until the monkey removes its mouth from the spout. Liquids are stored in plastic containers located outside the cage, above the level of the drinking spouts. Details of the drinking devices have previously been published (Henningfield and Meisch, 1977).

Smoking Device. A similar-sized spout made of stainless steel is used for smoking. The spout is open from the back so a coil of wire containing cocaine, heroin or other drugs can be inserted into the interior of the spout. The wire coil is coated with a precise amount of the drug that is to be smoked. This is done by dissolving the drug in 95% alcohol, dropping it on the coil *with a I ml syringe* and allowing it to evaporate. The end of the coil, distal to the monkey, is connected to electrical circuitry that heats it and volatilizes the drug. Inhalation responses on the smoking spout are registered by a vacuum switch. After 5 inhalations the coil of wire is heated, and can be inhaled on the fifth inhalation response. Details of the smoking device have been previously described (Carroll et al., 1990).

Control Equipment. Each monkeys' behavioral schedule is controlled by an IBM-compatible computer with a Med-PC interface (Med Associates, St. Albans, VT) that can control up to 12 chambers.

Drugs. Self-administration drugs such as cocaine, heroin, PCP, and methadone will be obtained from NIDA Research Triangle Institute, Research Triangle Park, NC). Ethanol will be purchased from the University of Minnesota Chemical Storehouse. Treatment drugs such as baclofen *and* bremazocine will be purchased from suppliers such as (Sigma-RBI, Sigma-Aldrich Chemical Co., St. Louis, MO). Treatment drugs will be dissolved in saline or vehicle immediately before use. Drugs used for liquid consumption will be mixed in tap water weekly in a concentrated stock solution, and then mixed daily to a specific concentration and stored at room temperature before use. Drugs that are used for smoking will be mixed every 3 or 4 days in 95% ethanol and stored in volumetric flasks with vacutainer tops. Amounts necessary for specific doses will be removed by a 1 ml syringe, dropped on nichrome wire coils in specific amounts for the weight of each monkey and allowed to evaporate for at least 24 hr on the coil before they are used in the smoking experiment. Coils will be weighed when dry on an analytical balance before and after loading with drug to determine that the precise dose is delivered.

Behavioral Procedures

Liquid-Reinforced responding: Most monkeys will have been previously trained to self-administer orally-delivered drugs; however, 9 new drug naive monkeys will be trained to self-administer drug (e.g., PCP) at a moderate concentration (e.g., 0.25 mg/ml). Drugs are initially presented in the daily water supply in the electronic drinking spouts during daily 3-hr sessions. Water is available at all other times. The daily food ration is given prior to the 3-hr session to induce drinking. The FR for liquid deliveries is then gradually increased from 1 to 2, 4 and 8. After behavior stabilizes at FR 8 the food is given after the session. Next, water is substituted at one drinking spout, so the monkeys have a concurrent choice between drug and water under FR 8 schedules. During this initiation process body weights are gradually reduced from free-feeding (100%) to 85%. These methods have been described in detail previously (Campbell et al., 1998; Carroll et al., 2000d); they are summarized in Table 2.

Table 2 Acquisition Procedure for New Monkeys and Experiment a(2b)

Step*	Liquid(s) Available	FR	Feeding Conditions
1	Water at both spouts	1	Food ad libitum
2	Drug at both spouts	1	Food ad libitum
3	Drug at both spouts	1	Food restricted, fed 30 min after session
4	Drug at both spouts	1	Food restricted, fed 30 min before session
5	Drug at both spouts	2	Food restricted, fed 30 min before session
6	Drug at both spouts	4	Food restricted, fed 30 min before session
7	Drug at both spouts	8	Food restricted, fed 30 min before session
8	Drug at both spouts	8	Food restricted, fed 30 min after session
9**	Drug and water	8	Food restricted, fed 30 min after session

* Each of the 9 conditions is held constant until 5 days of stable responding are obtained.

** Test for reinforcement = drug intake > water intake, $p<0.05$

In most experiments drug solutions or drug and concurrent water are available during daily 3-hr sessions, from 10:00 a.m. to 1:00 p.m. There is a 2 hr timeout before session (8:30-10:00 a.m.) and a 1.5-hr timeout after session from 1:00 to 2:30 p.m. During the timeout, liquids will be measured and changed, data will be recorded, stimulus lights will be turned off, and responding will have no programmed consequences. During the 1.5 hr timeout after sessions the monkeys are given their daily food allotment, and water is available from both spouts under FR 1 schedules during the 17.5 hr intersession period.

Smoke-reinforced responding: Smoking will be trained after the monkeys are first accustomed to self-administering water from the liquid apparatus. Jelly or peanut butter is placed on the smoking spout to initiate contact. Initially, each inhalation or sucking response results in a smoke delivery. When responding reliably occurs, the FR for smoke inhalations is increased to 5. Subsequently, a lever press response is added, starting with FR 1, and it can gradually be increased to 2, 4, 8, 16, 32, 64 and higher depending on the specific experimental design. Ten smoke deliveries are available within a 3-hr period each day, and each one is followed

by a 15 min timeout when all stimulus lights are extinguished and responding has no consequences. There is a 1-hr timeout before and after the smoking session when data are recorded and liquid intake is measured. One liquid spout, usually containing water, is operable under an FR 1 schedule during the session and intersession period. A ial consists of a lever-press requirement (FR) and is signaled by a red light over the lever. A red light over the lever is illuminated signaling the start of a smoking trial When the lever press FR is completed, a green blinking light over the smoking device is illuminated, and it remains on until 5 inhalation responses are made and the coil is heated, releasing the smoke. If the response requirements are not met in 2 consecutive trials, the session is terminated, and a timeout will remain in effect for the remainder of the 3-hr session.

Progressive Ratio (PR) Schedule: PR schedules are used to evaluate how hard an animal will work for drug reinforcement or as an estimate of its reinforcing efficacy. The FR is increased for each successive reinforcer delivery, and the highest FR completed before responding ceases is the break point which is often used as a measure of relative reinforcing efficacy (Griffiths et al., 1979; Katz, 1990; Stafford et al., 1998). In the proposed drug self-administration procedure PR schedules will be used in some experiments to compare reinforcing effects under different conditions. They can be used either alone (smoking) or concurrently (oral) to compare 2 doses of a drug, 2 drugs or drug and nondrug reinforcers. In the drinking PR, the response is a lip contact on the drinking spout, and in the smoking PR, it is a lever press. The drinking PR begins at 8 and increases in log (base 2) and half log steps from 8 to 16, 32, 64, 128, 178, 256, 356, 512, 712, 1024, 1424, 2848, and 4096. After response completion, a fixed amount of liquid deliveries (e.g., 20) is available under an FR 1 schedule to be consumed within 10 min. The PR steps are the same for smoking, except they start at 64. When each response requirement is met, one smoke delivery is available, and there is a 15 min timeout after each delivery.

Demand Curve Analysis-Behavioral Economics: Another way to quantify and compare the reinforcing efficacy of a drug under different experimental conditions is to construct a demand curve. Consumption is plotted as a function of unit price (responses/mg) often determined by FR schedules (unit price=FR). The measures of interest on a demand curve are its elasticity of demand (slope) or susceptibility to change (slopes with a more negative value than -1 are elastic, while those between 0 and -1 are inelastic), and the unit price at which maximum esponding occurs (P_{MAX}). A demand curve is another way of estimating how resistant an animal is to increases in price, or what is the most it will "pay" for the drug, and it is analogous to a PR schedule (Bickel et al., 2000). However, under some conditions, behavioral economic procedures maintain behavior more reliably and over a longer period of time than a PR schedule because responding does not reach the point where it completely stops. To measure demand, consumption of the drug is measured over about 6 unit prices (FR's) ranging from those that minimally affect consumption to those that markedly decrease it. In the proposed experiments with liquids and food the FR values will be 4, 8, 16, 32, 64 and 128. When the smoking procedure is used the FRs are 32, 64, 128, 256, 512 and 1024. FR values are tested in nonsystematic order that is counterbalanced across monkeys.

Determining Phase of Menstrual Cycle: The menstrual cycle of rhesus monkeys is 28-days and is similar to humans. The 3 major phases are the follicular phase beginning on the first day of menstruation when estrogen levels are rising *and progesterone is low,* the periovulatory phase when estrogen peaks and ovulation occurs, and the luteal phase when high estrogen levels rapidly decrease *and progesterone is high.* These phases can be verified by *changes in estrogen and progesterone levels in the blood. Increased progesterone levels during the luteal phase will serve as an indicator that the menstrual cycle was ovulatory.* The phases will be determined by *taking blood samples during the intersession periods every 2 or 3 days.* Monkeys will be trained to present *their leg* for *blood draws* by rewarding them with a small treat. *Initially they may have to be anesthetized to obtain blood samples. Samples will be taken after the daily session data are collected so any stress from the procedure will not influence the results.* The color of the perineal area will *also* be scaled on a 1-6 scale according to the methods of Baulu (1976) and Vandenbergh (1965) whereby 1=no redness and 6=brightest red. The color changes correspond well with phases and hormonal changes, and *will be used as another method of monitoring the cycles. Testosterone levels will also be determined in males by drawing blood samples 3 times a week.*

Data Analysis: The number of subjects per experiment was determined by previous sample sizes that had sufficient power to achieve statistically-significant results. Much of the oral drug self-administration work and almost all of the smoking studies have been conducted in this laboratory. Thus, it is possible to provide reasonable estimates of the number of subjects needed in each experiment. Many of the experiments are designed to use a

within-subjects or repeated-measures protocol. The primary dependent measures and specific data analysis strategies are described for the specific experimental designs. When conditions are held constant until behavior is stable, that is defined by 4 or 5 days during which there is no steadily increasing or decreasing trend in the ependent measures. The level of significance that will be accepted for all statistical analyses is $p<0.05$, except where noted for *multiple* subsequent comparisons ($p<0.01$). Statistical analyses will be generated using GB-Stat, StatView, (Abacus Concepts Inc, Berkeley, CA) Super ANOVA and Sigmastat (Jandel Scientific, CA) software. Linear regression analyses on behavioral economic demand curves (slopes) will be calculated with CA-Cricket Graph III (Computer Associates International).

2. Specific Experiments

The basic themes of these experiments are vulnerability factors in drug abuse such as sex, hormonal status and amount of drug access, and the effect of behavioral and pharmacological treatments *as well as their combination.* Other goals of the proposed research are to use behavioral economic measures to evaluate the relative reinforcing efficacy of the drugs of abuse under various experimental conditions, and to evaluate sex differences in treatment *The proposed work takes place during the maintenance phase of drug self-administration. Acquisition studies are not proposed because only 9 new naive monkeys will be added to the existing group. It would be interesting to incorporate a model of reinstatement of extinguished drug-seeking behavior, as this is a phase of addiction when humans are most vulnerable to relapse. However, in this laboratory, reinstatement models have not worked well, and it is likely the case in other primate laboratories as well, as there are only two published studies (e.g., Kautz and Ator, 1995; Khroyan et al., 2000; Spealman et al., 1999) of reinstatement in nonhuman primates. In contrast, nonhuman primates (vs. rats) are an excellent model for behavioral economic analyses, thus the proposed experiments incorporate this feature in most experiments.* The 10 proposed experiments are presented according to the following outline with the (a)s, and (b)s *referring to the 10 specific* experiments.

a. Vulnerability to drug self-administration
    (1) Sex and hormonal status
        (a) progressive ratio
        (b) demand (behavioral economic measure)
    (2) Access conditions - short vs long in males and females
        (a) change in reinforcing efficacy, set point
        (b) acquisition of new drug (methadone) self-administration
b. Treatment strategies
    (1) Behavioral interventions
        (a) alternative reinforcers in males and females
        (b) substitution of drug and nondrug reinforcers (in males)
    (2) Pharmacological interventions
        (a) baclofen, dose effect and demand for drug in males and females
        (b) bremazocine, dose effect and demand for drug in males and females
    (3) Combined behavioral and pharmacological vs. each treatment alone.
        (a) alternative reinforcers combined with medication vs. single treatments in males and females
        (b) open vs. closed economy in males and females

a. Vulnerability to drug abuse

a(1) Sex and hormonal status

a(1a) Self-administration of orally-delivered PCP, and saccharin and smoked cocaine base and heroin under PR schedules: Effect of sex and hormonal status. Initial results with rhesus monkeys indicate that females more readily self-administer PCP than males (Carroll et al., 2000d). While both males and females consumed similar amounts, females consumed more (mg/kg) since they weigh less than males. It is the purpose of the proposed experiment to determine whether the reinforcing effects of PCP, heroin, cocaine, food and saccharin are greater in females than in males and how they are influenced by hormonal status. Their drug-maintained performance will be compared under PR schedules as described in the General Methods.

Seven female and 7 male rhesus monkeys (Group 1, see Table 9) will be trained to self-admininster PCP, and saccharin according to the oral methods described in General Methods, and they will also be trained to smoke cocaine base and heroin. The female monkeys will be tested with the PR schedule at each phase of the menstrual ycle (See General Methods), and data will be summarized for each stage (early, mid, late) of each phase. The ıales will be yoked to the females with data used from the same days to balance the pattern of testing. The experimental design is outlined in the left portion of Table 3. It is expected that the amount of self-administered drug consumed over a 3-hr session will not interfere with the menstrual cycle or estrogen levels as it does with chronic access to drug (Craft et al., 1999; Mello et al., 1983, 1997). If this is a problem, adjustments in consumption will be made. *Another factor which may limit interference of drug self-administration with the menstrual cycle is that periods of time will be spent off drugs when food and saccharin comparisons are made. This will allow for a within-subject assessment of hormonal cycles with and without drug self-administration. Blood samples that are taken during the intersession periods will be analyzed with respect to estrogen and progesterone levels in females and testosterone levels in males. Blood samples will also be analyzed at different time periods postsession (0, 30 min and 1 hr) to compare plasma levels of the abused drug in males and females to determine whether sex and hormonal differences in intake (mg/kg) are due to pharmacokinetic factors. Inclusion of the saccharin condition will enable us to determine whether sex and hormonal effects are selectively related to drug self-administration or whether there general differences in females such as increased locomotor activity which has been reported in rats (Cronan et al., 1985).*

Dependent Measures and Data Analysis: Responses, break point on the PR schedule, number of liquid deliveries consumed and mg/kg intake will be compared across males and females at 3 phases of the menstrual cycle (follicular, periovulatory and luteal). *Blood samples will be used to verify estrogen and progesterone levels throughout the menstrual cycle, and self-administration behavior will be analyzed with respect to these hormone levels.* Repeated measures factorial analyses of variance (ANOVAs) will be performed separately for each dependent measure to assess the main effects of sex, hormonal status, and the repeated measure (drugs and phase) as well as the interactions. Linear contrasts ($p<0.01$) or Bonferroni-corrected post hoc t-tests between males and females at each phase will be made to further examine main effects.

a(1b) A behavioral-economic analysis of demand for orally-delivered PCP, saccharin and smoked cocaine ase and heroin: Effect of sex. Another method for evaluating the relative reinforcing efficacy of drug in males vs. female monkeys is the behavioral economic analysis of demand (Bickel et al., 2000). The purpose of this experiment is to construct demand functions with cocaine, heroin, PCP and saccharin, and compare males to females in terms of the intensity and elasticity of demand and P_{MAX} *(See General Methods).*

Seven female and 7 male rhesus monkeys (Group 1) will participate in this experiment when they have finished Experiment a(1a). The demand curve will be generated by using 6 FR values that vary depending on whether the drug is orally self-administered or smoked (See General Methods). The order of testing the self-administered substance (cocaine, heroin, PCP or saccharin) and the FR values will be nonsystematically determined and counterbalanced across monkeys. *Each FR will be held constant until at least 4 days of stable data (no increasing or decreasing trends) are obtained. Again, saccharin data will assist in determining whether sex differences are due to general differences in operant levels of responding or specifically to the reinforcing effects of drugs.* The experimental design is outlined in the center shaded portion of Table 3.

Table 3 Sex and Hormonal Status Experiments a(1a), a(1b) and b(1a)

	Exp a(1a) PR Schedule					Exp a(1b) Demand Curves FR 4, 8, 16, 32, 64, 128				Exp b(1a)		
	Cocaine	Heroin	PCP	Sacc		cocaine + water	heroin + water	PCP + water	sacc + water	cocaine + sacc	heroin + sacc	PCP + sacc
Males N=7					*Males N=7*							
Females N=7 follicular					*Females N=7*							
ovulatory												
luteal												

Dependent Measures and Data Analysis: The dependent measures for this experiment will be responses, deliveries, and drug intake (mg/kg). Under each drug condition and for males and females at the 3 menstrual phases, as well as specific (early, mid, late) stages within each phase, demand curves will be plotted (consumption FR) on a log-log scale. Point-to-point slopes will be obtained using linear regression and averaged, and overall slope will also be assessed. P_{max} values (point at which maximum responding occurs) will be obtained using SuperANOVA software according to procedures described by Hursh (1991). The dependent measures will also be compared using factorial ANOVAs with FR as a repeated measure and male vs. female as a between group factor. Linear contrasts will be used to further identify significant paired comparisons.

a(2) Access conditions

a(2a) Short- vs long-daily access to drug self-administration: Change in homeostatic set point and reinforcing efficacy. One of the more pressing goals of drug abuse research cited by NIDA (1999) is to obtain a full understanding of the transition from drug use to drug addiction and to design prevention and treatment approaches for different points on this trajectory. Models for the escalation of drug use exist in rats (e.g., Ahmed and Koob, 1998, 1999); however, attempts to allow drug use to escalate by unlimited access in monkeys have had adverse effects (Johanson and Balster, 1976; Aigner and Balster, 1978). A goal of the proposed experiment is to develop a safe model in rhesus monkeys to analyze the transition between drug use and addiction. These studies will be patterned after the rat work cited above, but they will extend the outcome measures by using PR schedules and behavioral economics to characterize the changes in reinforcing efficacy of a drug due to increased access.

Twenty male and 14 female rhesus monkeys will be used in this experiment. Groups 1, 2 and 3 will be used (See Table 9). Initially, *all* groups will be allowed 1 hr of continuous access to PCP and concurrent water under an FR 1 schedule for 30 days. Access to PCP will then be increased to 6 hr in one group, kept the same (1 hr) in the other groups *and increased to 12 hr in one group of 6 males (Group 3). There will not be enough female monkeys to complete the 3 access conditions, thus females will be tested at 1 and 6 hr, and only males at 12 hr.* This condition will also be held in effect for 30 days. After this escalation phase, both groups will be returned to the 1-hr sessions for 60 days. During this post escalation phase, a concentration effect function will be obtained by substituting a different concentration for 2 days every 5 days. A total of 5 concentrations will be studied (0.06, 0.12, 0.25, 0.5 and 1 mg/ml) in mixed order and counterbalanced across monkeys.

The next part of this experiment will be to compare the reinforcing efficacy of PCP in these 2 groups by using a PR schedule and a behavioral economic analysis of demand *using* procedures described in General Methods. A 20-day period of differential access *(1 vs. 6 vs. 12 hr)* will be reinstated to recapture the baseline. Next, the session length will be changed to 1 hr for *all* groups and a PR schedule will be examined for 5 days with 5 different concentrations available for 2 days each (0.06, 0.12, 0.25, 0.5 and 1 mg/ml). The differential access condition will then be reinstated for 20 days, and then session length will be returned to 1 hr for all groups. Over the last 30-day period a demand curve will be generated by using 6 FR values for 4 days each. The design of this experiment is summarized in Table 4. *An alternative interpretation of escalating drug intake as in the case of the Ahmed and Koob study, (1998, 1999), is that over time animals gain weight, and increased drug intake is necessary to maintain a constant dose (mg/kg) when the unit doses are constant (e.g., 0.25 mg). The monkeys used in the proposed study will be adults, and body weights will be monitored to ensure that they remain stable.*

Table 4 PCP Access Conditions

	Exp a(2a)							Exp a (2b)	
	Set Point Determination		Dose Response (0.06, 0.12, 0.25, 0.5, 1.0)	Reinforcing Efficacy				Baseline	Methadone Acquisition
Session	30 days	30 days	30 days	Baseline 20 days	PR 10 days	Baseline 20 days	Demand Curve	30 days	60 days
Type * Length	short 1 hr	short 1 hr	Short 1 hr	short 1 hr	short 1 hr	short 1 hr	30 days short 1 hr	short 1 hr	medium 3 hr
Type * Length	short 1 hr	long 6 hr	Short 1 hr	long 6 hr	short 1 hr	long 6 hr	long 6 hr	long 6 hr	medium 3 hr
Type ** Length	short 1 hr	longer 12 hr	Short 1 hr	longer 12 hr	short 1 hr	longer 12 hr	longer 12 hr	longer 12 hr	medium 3 hr

* This condition will be conducted with 7 males and 7 females

** This condition will be conducted with 6 males

Dependent Measures and Data Analysis. During the initial set point determination the dependent measures will be the number of responses, deliveries and PCP intake (mg/kg). Mixed ANOVAs with between-group factors (*length of access, male vs. female*) and within-group factors (pre-escalation (*1 hr*), escalation *6 hr, 12 hr)*, post-escalation *(1 hr),* will be used to compare the effects of differential access at a fixed (0.25 mg/ml) PCP concentration. Post-hoc comparisons will be made with linear contrasts or Bonferroni-corrected t-tests. The concentration-response function will then be analyzed with mixed ANOVAs with concentration as the repeated measure and length of access and sex as the between-subjects factors. Similar subsequent tests will be conducted if main effects are significant. The determination of reinforcing efficacy will be done using the PR schedule and demand curve analysis; thus, dependent measures and statistical procedures will be similar to those described for Experiments a(1a) and b(1a), respectively.

a(2b) Acquisition of new drug self-administration after short vs. long daily exposure to another drug. The purpose of this experiment is to examine the question: does escalation of self-administration of one drug make the animals more vulnerable to increased self-administration of a second drug *that has not previously been self-administered*? The 3 groups that showed controlled intake vs. escalated intake of PCP *in the previous experiment* will be exposed to a second drug that they have not previously experienced (e.g., methadone), and the rate of acquisition as well as the level of stable intake will be compared.

The same 34 monkeys from Groups 1, 2 and 3 that were used in Experiment a(2a) will participate in this experiment. When they have finished the previous experiment, they will be placed on differential (1 vs. 6 *vs.12 hr)* access to PCP for 30 days. Subsequently, methadone (0.8 mg/ml) will be substituted for PCP, because it is orally self-administered in monkeys (Wang et al., 1999), and a modified version (beginning with Step 2) of the acquisition procedure outlined in Table 2 for PCP will be implemented.

Dependent measures and Data Analysis. The dependent measures will be lip-contact responses, methadone and water intake (ml and mg/kg). Before the test for reinforcement (Step 9, Table 2) under food restriction conditions of the acquisition procedure, *a mixed ANOVA will be used with FR changes as the repeated measure and length of access and sex as the between-group factors.* During the test for reinforcement *Bonferroni-corrected* paired t-tests will be used to determine whether methadone intake is significantly greater than water intake.

b. Treatment Strategies

b(1) Behavioral interventions

b(1a) Effects of a nondrug reinforcer on the demand for PCP, cocaine and heroin: Effect of sex. While more evidence is accumulating regarding sex differences in drug abuse in humans as well as animals, data are lacking regarding the effects of various drug abuse treatment methods in males vs. females. This is also a pressing objective that NIDA (1999) has outlined for further research. The goal of this experiment is to compare the effect of a nondrug reinforcer, saccharin, on cocaine, heroin and PCP-maintained behavior in male and female monkeys.

Seven male and 7 female rhesus monkeys (Group 1) will be used in this experiment, which is an extension of Experiment a(2b). The demand curves that were generated with drug and concurrent water will also be determined with concurrently available saccharin. Saccharin will be used at a concentration (0.3% wt/vol) that has been shown to suppress cocaine- (Comer et al., 1994) and PCP-reinforced (Carroll 1985, Rodefer and Carroll, 1997) behavior. The 6 conditions shown in the center shaded and right portion of Table 3 will be presented in nonsystematic order and counterbalanced across monkeys: cocaine + water, cocaine + sacc, heroin + water, heroin + sacc, PCP + water, PCP + sacc. Each FR value will be held in effect *until 4 days of stable behavior are obtained. A sacc + water condition will also be included, as shown in the striped portion of Table 3, to compare the amount of saccharin and water consumed in males vs. females. As in Experiment a(1b) males will be yoked to the females to control for duration and pattern of drug access.*

Dependent Measures and Data Analysis: The dependent measures and data analysis will be the same as indicated for Experiment a(1b).

b(1b) Substitution of drug and nondrug reinforcers for self-administered drugs. This experiment addresses the basic question of how well a drug or nondrug substance substitutes for the reinforcing effects of a drug of abuse. Behavioral economic analyses of the demand for drug are able to quantify these interreinforcer or polydrug

relationships. When the price of drug is increased, consumption decreases. When an alternative drug or substance is available at a fixed-price, and its consumption increases as the demand for the original substance decreases (due to increased price), the second substance functions as a *substitute*. Substances that will be self-administered and also tested as substitutes for other drugs will be smoked cocaine and heroin, and orally-delivered ethanol, PCP and saccharin. Ethanol will be used in this experiment because it is often used with other drugs and substituted when a user terminates use of another drug (NIDA, 1991).

Six male rhesus monkeys (Group 3) will be used in this experiment. *The lab is shifting from all males to equal numbers of males and females. However, there will still be 6 more males than females, and not enough females to examine sex differences in each of the proposed experiments. Data from this group will be used to compare behavioral economic data to that obtained with males in previous studies.* They will have had recent experience with short and long periods of drug access [Experiment a(2a)] and acquisition of methadone self-administration [Experiment a(2b)] as shown in Table 9. The experimental design is outlined in Table 5. There are 20 conditions in which one drug or saccharin will be tested as a substitute for another drug or saccharin. A demand curve will be generated by varying the FR for the self-administered drug, and the potential substitute drug or saccharin will be available at a fixed FR. The fixed FR will be 8 for ethanol, 16 for PCP and saccharin and 128 for cocaine and heroin. The 20 conditions will be run in nonsystemmatic order and counterbalanced across monkeys.

Dependent Measures and Data Analyses: Demand curves will be constructed as described for Experiment a(1b) using the same dependent measures, for the substances with a varied FR. Cross-price elasticity regression coefficients will also be obtained for the substances with fixed FR values. When plotted on log-log coordinates a cross-price elasticity coefficient $\geq$ 0.2 will be defined as a substitute, $\leq$ -0.2 will be defined as a complement, and between 0.2 and -0.2 will be defined as an independent. Point-by-point slopes as well as the overall best-fitting line will be calculated. Changes in consumption across FR values will be analyzed by repeated measures ANOVAs, and Bonferroni-corrected matched pairs t-tests will be used to further analyze overall main effects.

Table 5 Substitution of Drug and Nondrug Reinforcers

Exp b(1b)

Varied FR (4, 8, 16, 32, 64, 128) For:		Fixed FR (16) for Concurrent:				
		Cocaine	Ethanol	Heroin	PCP	Saccharin
✓	Cocaine		✓			✓
	Ethanol					
	Heroin					✓
✓	PCP					
	Saccharin					

6 male monkeys will be used in all 20 conditions indicated by open blocks

b(2) Pharmacological interventions

b(2a) Effects of baclofen on the demand for self-administered cocaine, heroin, PCP, food and saccharin: Effect of sex. The purpose of this experiment is to compare the effects of baclofen, a $GABA_B$ receptor agonist, on behavior maintained by drug and nondrug reinforcers in male and female rhesus monkeys. This work will extend the findings that baclofen decreases i.v. cocaine self-administration in rats (Campell and Carroll, 2000a; Campbell et al., 2000a) and cocaine use and craving in humans (Ling et al., 1998) to monkeys, other drugs of abuse, and other routes of administration. It is hypothesized that baclofen will suppress drug- and saccharin-maintained behavior, but it will have a minimal effect on food-maintained behavior. *Baclofen currently appears to be a promising treatment drug, however, another medication may be substituted if one that is more selective for suppressing drug self-administration and has minimal side effects is available.*

Seven male and 7 female rhesus monkeys (Group 2) will be trained to self-administer smoked cocaine base (1 mg/kg) and heroin (0.1 mg/kg), and orally-delivered PCP (0.25 mg/ml), food (10 g) and saccharin (0.3 % w/v) according to procedures described in the General Methods section. Food- and saccharin-maintained behavior will be examined as controls for drug-selective effects. While each substance is available for self-administration, 3 doses of baclofen (0.5, 1.25 and 2.5 mg/kg) and saline will be administered i.m. 15 min prior to the 3-hr self-administration session for 21 consecutive days. *If increasing or decreasing trends in the suppression of drug self-administration occur within the 21-day treatment period, the length of treatment will be extended to more closely approximate the human situation and fully characterize the time course of treatment effects. If there are no changes in the magnitude of the treatment effect over the 21 days using several doses and under different self-administration conditions, the duration of treatment may be shortened to 5-10 days using the last 5 days as a*

sample of stable behavior. Three concentrations (PCP) or doses (cocaine, heroin) of each drug will also be tested at one (optimal) dose for the treatment drug. The order of testing the self-administered substances, and baclofen doses will be counterbalanced across monkeys. The dose or concentration used for the self-administered drug will be selected from the middle of the range of effective reinforcing doses/concentrations. The baclofen doses were based on the rat and human studies cited above. The FR value will be held constant at 16 for food and drinking and 128 for smoking, values that are sensitive to behavioral and pharmacological treatments. The left side of *the shaded portion of* Table 6 illustrates the experimental design. *Under each baclofen dose condition, blood samples will be taken during intersession to compare effects on the self-administered drug in males vs. females. Females may be more or less sensitive to baclofen than males. Thus, it is necessary to compare dose response curves across males and females and to observe any physical signs indicating side effects.*

Table 6 Pharmacological Interventions

Self-administered substance	Dose	Exp b(2a) Dose effect, saline + 3 doses - - Pretreatment Drug - - Baclofen Female	Exp b(2a) Baclofen Male	b(2b) Bremazocine Female	b(2b) Bremazocine Male	Exp b(2a) Demand curve FR 4, 8, 16, 32, 64, 128 - - Pretreatment Drug - - Baclofen Female	Exp b(2a) Baclofen Male	b(2b) Bremazocine Female	b(2b) Bremazocine Male
Cocaine	Low Med High								
Heroin	Low Med High								
PCP	Low Med High								
Saccharin									
Food									

In the second phase of this experiment the effect of baclofen on the demand for cocaine, heroin, PCP, food and saccharin will be evaluated by using an effective dose of baclofen that was determined by the dose effect function, and then varying the FR for the self-administered substance to construct demand curves. The demand functions will be obtained according to the procedures described in General Methods. The drug dose and amount of food or saccharin will be selected such that the demand curves (e.g., P_{max}) are similar for the 5 substances. Baclofen will be administered at each of the FR requirements for *at least* 5 days *or until behavior stabilizes,*, and the preceding 5-day period will serve as a nontreatment control. FR values will be tested in nonsystematic order and counterbalanced across monkeys. The *left side of the unshaded* portion of Table 6 summarizes the design of Experiment b(2a).

Dependent Measures and Data analysis. Responses, deliveries, volume, and mg/kg consumed from the 5 days of stable behavior before treatment will be compared to the last 5 days of baclofen treatment. Behavior will be allowed to stabilize for 5 consecutive days before changes in the experimental conditions are made. Stability is defined as no steadily increasing or decreasing trend in the dependent measure over 5 days. Dose effect comparisons will be made using repeated measures (treatment dose, and/or self-administration dose) ANOVAs and subsequent comparisons as described for Experiment a(2a). Similarly the demand curve analysis will be conducted as described for Experiment a(1b).

b(2b) Effects of bremazocine on the demand for self-admininstered cocaine, heroin, PCP, food and saccharin: Effect of sex. The goals of this experiment are to compare the effects of bremazocine, a kappa receptor agonist, on behavior maintained by drug and nondrug reinforcers in male and female rhesus monkeys. Preliminary data (Study 3) indicate that bremazocine dose-dependently reduces cocaine, PCP and saccharin self-administration in male monkeys, but it has less of an effect on ethanol- and food-maintained behavior. This experiment will extend the research to other drugs (e.g., heroin), routes of administration (smoking), and it will determine whether there are sex differences in medication effects.

The design of this experiment is summarized in Table 6 *(right side of shaded portion)*, and the procedure is similar to that described for Experiment b(2a). Seven male and 7 female rhesus monkeys (Group 2) will be trained to self-administer smoked cocaine base (1 mg/ml) and heroin (0.1 mg/ml) and orally-delivered PCP (0.25 mg/ml), saccharin (0.3% w/v) and food with water concurrently available as described in the General Methods section. While each substance is available for self-administration, 3 doses of bremazocine (0.32, 1 or 2.5 mg/kg) and saline will be administered i.m. 15 min prior to the 3-hr self-administration session for *21* consecutive days. *As in the case*

of baclofen, the treatment period will be extended if consistent trends in the treatment effect occur over time, and it will be shortened to 5-10 days if treatment effects are stable over the 21 days, and under several dose conditions. Three concentrations (PCP) or doses (cocaine, heroin) of each drug will be tested at one treatment drug dose. The order of testing the self-administered substances, bremazocine and dose will be counterbalanced across monkeys. The bremazocine doses were based on previous research (e.g., Mello and Negus, 1998; Nestby et al., 1999) and preliminary data (Study 3) obtained with rhesus monkeys. The FR value will be held constant at 16 for food and drinking and 128 for smoking, values that are sensitive to behavioral and pharmacological treatments.

In a second phase of this experiment the effect of bremazocine on the demand for cocaine, heroin, PCP food and saccharin will be evaluated by using an effective dose of bremazocine (determined from the first phase of the experiment) and then varying the FR for the self-administered substance to construct demand curves. The amount (mg/ml or mg/kg) of the self-administered substances will be selected to generate similar demand curves (e.g., P_{max}). The demand function will be obtained according to the procedures described in the General Methods. Bremazocine will be administered at each of the FR requirements for 7 days, and the preceding 5-day period will serve as a nontreatment control. FR values will be tested in nonsystematic order and counterbalanced across monkeys. *Blood samples will be collected during intersession at each of the bremazocine dose levels to compare effects on plasma drug levels in males and females. The right side of the unshaded part of Table 7 summarizes the procedure.*

Dependent Measures and Data Analyses. This will be the same as described for experiment b(2a).

b(3) Behavioral-Pharmacological Treatment Interactions in males and females

b(3a) Effects of medication pretreatment on the suppression of cocaine, heroin and PCP self-administration by nondrug alternative reinforcers in male and female monkeys. Initial work with buprenorphine as a treatment drug (shown in Table 1) indicates that the medication is more effective when combined with a behavioral treatment (i.e., access to a nondrug alternative reinforcer) (Bickel et al., 1997a,b; Bickel and Marsch, 2000; Carroll et al., 2000a). One goal of the present proposal in testing 2 proposed medications in *male and female* monkeys is to determine whether they are more effective in combination with the behavioral, nondrug reinforcer treatment.

The experimental design for this experiment is shown in Table 7. *Seven male and 7 female rhesus monkeys will be used (Group 2)* as shown in Table 9. Baclofen and bremazocine will be the treatment drugs used at doses that produced moderate effects in previous experiments. Concurrent availability of a palatable saccharin (0.3% w/v) solution will be the nondrug reinforcer. These treatments will be tested separately and in combination for their effect on the demand for smoked cocaine- and heroin- and oral PCP. Demand curves will be constructed for each treatment condition as described in the General Methods. It would have been efficient to use monkeys form Groups 1 and 2 that had the behavioral [Group 1, b(1a)] or medication [Group 3, b(2a,b,c)] treatment alone, but that would not fit within the 5 year time line (Table 9), nor would it not allow treatments and self-administered drugs to be counterbalanced across monkeys as they will be in this experiment.

Table 7 Combined Behavioral and Pharmacological Treatments

Exp b(3a)

Pretreatment Conditions	Self Administered Drug: Cocaine		Heroin		PCP	
	males	females	males	females	males	females
Baclofen						
Saccharin						
Baclofen + Saccharin						
Bremazocine						
Saccharin						
Bremazocine + Saccharin						

* for each of the 18 treatment conditons a demand curve will be obtained by using FR 4, 8, 16, 32, 64, 128

Table 8 Open vs. Closed Economy

Exp b(3b)

Self-administered Drug	Economy		Pretreatment Drug: Baclofen (saline + 3 doses)	Bremazocine (saline + 3 doses)
Cocaine	open	M		
		F		
	closed	M		
		F		
Heroin	open	M		
		F		
	closed	M		
		F		
PCP	open	M		
		F		
	closed	M		
		F		

* a PR schedule will be used. Five days no treatment baseline, 5-7 days Drug pretreatment, 5 days post treatment. M=male F=female

Dependent Measures and Data Anlaysis: Dependent measures for each cell shown in Table 7 will be mean responses, deliveries and intake (mg/kg) for 7 *male and 7 female* monkeys over the last 5 sessions of stable behavior. Separate *mixed* ANOVAs will be performed to compare drug treatment, *the alternative reinforcer* treatment, or the combined treatments, *as repeated measures* for each self-administered drug *with sex as the between groups factor. A priori* treatment effects will be analyzed by simple contrasts. Demand curve analyses will be conducted as described for Experiment a(1b). Isobolographic analyses will be used to determine whether combined treatments are additive, infraadditive or supraadditive.

b(3b) Effects of drug pretreatment on drug self-administration in an open vs. closed economy in male and female monkeys. The economic condition under which drug self-administration occurs has a considerable influence on the extent to which behavioral and pharmacological treatments are effective. One variable that is important to drug abuse treatment is whether the economy in which the drug is available is open or closed. A *closed economy* is one in which the earned commodities are all that is available to the animal, and an *open economy* is when earned commodities are supplemented by the experimenter noncontingently upon the animal's behavior. When naltrexone pretreatments were given to monkeys in a *closed economy* for ethanol, saccharin, PCP or food, a reduction in intake was found only with ethanol and saccharin, suggesting an opioid-mediated effect. When an *open economy* was created by supplementing the animals' earned access with free access to drug or dietary items after their self-administration session, naltrexone reduced the intake of all 4 substances, suggesting a nonselective effect. The open economy further enhanced the reductions in ethanol and saccharin self-administration (Carroll et al., 2000b). There are clinical findings that support this principle (O'Malley et al., 1996, Volpicelli et al., 1995).

In the proposed research, this procedure will be extended to treatment drugs other than naltrexone, such as baclofen or bremazocine, to female monkeys and to the smoking route of self-administration. The open vs. closed economy comparisons will be made with cocaine, heroin or PCP functioning as reinforcers. Seven males and 7 females will be compared under each of these conditions. The monkeys will be from Group 1 as indicated in Table 9. The experimental design to be used in this experiment is outlined in Table 8. The procedure used to study the effect of open vs. closed economies on drug pretreatment efficacy has been previously described (Carroll et al., 2000b). It is identical to that described for the dose effect portion of the 3 pretreatment studies described above b(2a), b(2b), b(2c), Table 6), whereby the treatment drug is injected before the 3-hr self-administration session. The FR schedule will be held constant at FR 16 for liquid drug or food and FR 128 for smoked cocaine or heroin. The self-administered drug dose *or concentration* will be held constant at an amount used to equalize demand curves. Treatment drugs will be baclofen *and* bremazocine at the 3 doses described above, and saline. Each drug or saline will be administered *until 5 days of stable behavior are obtained*, and the 5 days before injections will serve as the nondrug baseline. To create an open economy, the substance that is self-administered under an FR 16 schedule is also given later in the day. In the case of drugs or liquid, a fixed number of deliveries are freely available under FR 1 schedule. The open-economy condition will be varied by allowing postsession access to 1, 2 or 3 times the amount the animal earned during the 3-hr session. The amount will be proportional to what the animal self-administered because there is wide variety in intake among male monkeys (Carroll et al., 2000d), and there is expected to be variation in intake between males and females. Figure 7 summarizes the daily sequence of earned vs. supplemental commodities.

Dependent Meaures and Data Analysis: Mean responses, deliveries, mg/kg and break points will be calculated for each drug, type of economy, and in males and females. ANOVAs with between subjects (sex) and repeated measures (economy, treatment drug dose) will be used to analyze the results of this experiment. Posthoc comparisons will be made using Bonferroni-corrected t-tests or the Student-Newman-Keuls Method.

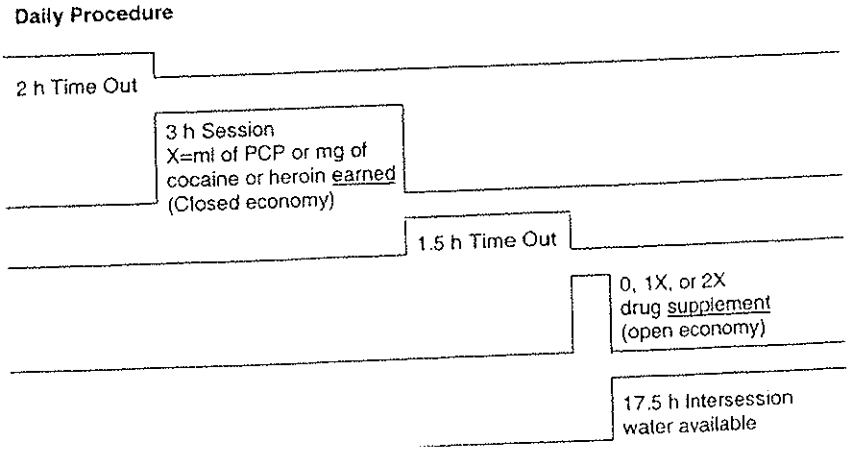
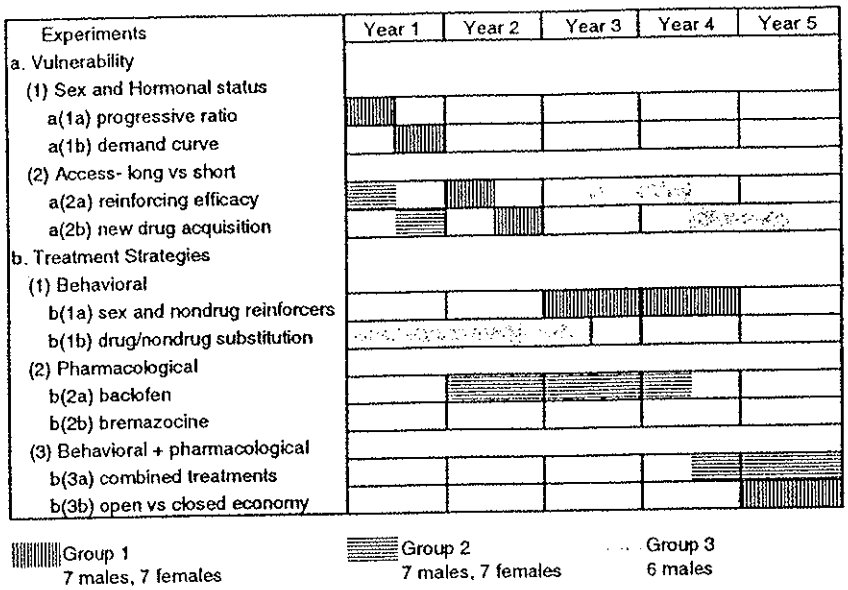


Figure 7

3. Time Line

The filled portions in Table 9 indicate the times when specific experiments will be conducted, and the groups (male and female) that will participate in each experiment.

Table 9



E. Human Subjects

None

F. Vertebrate Animals

1. Description of proposed use of animals

A total of 34 rhesus monkeys (*Macaca mulatta*) will be used in the proposed experiments. Fourteen will be female and 20 will be male. Over the 5-year period 9 existing monkeys in the colony of 34 will be replaced by 9 new drug-naive monkeys (7 females and 2 males) ranging in age from 4-6 years. There are 3 older animals in the colony (20-30 years) that will need to be replaced. There are currently 7 females, and a total of 14 will allow for 2 simultaneous experiments, as most group comparisons are done with 6-7 animals per group. It is necessary to have a larger group of females in order to conduct several simultaneous experiments on sex differences. The total number of monkeys never exceeds 34. It is also important to add drug naive animals to the group and compare their results with drug-experienced animals, *although* drug history is not a factor in well-trained animals that have stabilized levels of drug self-administration.

The monkeys live in their experimental test chambers, and stimulus lights on one wall indicate when experimental conditions are in effect. Three experimental rooms house the monkeys with 8, 12, and 14 animals in the respective rooms. There are panels attached to one side wall of the stainless steel chambers which contain levers for food-reinforced responding, and a food receptacle, 2 lip-operated drinking devices or 1 smoking device and 1 drinking device. Reservoirs that contain the liquids and circuitry that controls the smoking and drinking devices is attached on the outside of the cage wall out of the monkey's reach. Thus, the monkeys' access to foods and liquids is automated, but they are manually given food and water if the apparatus is under repair.

2. Justification

Monkeys were chosen for these experiments because the proposed models of drug abuse have been specifically developed for monkeys. For instance, monkeys are more suitable than other species (e.g., rats) for smoking drugs because their pulmonary system absorbs the particles. The particles are too large for rat alveolar tissue (Snyder et al., 1988). Also, monkeys orally self-administer bitter tasting drug solutions, while rats avoid bitter tastes. Ethanol and potent opioid solutions are the only drugs that are readily self-administered by rats. It is also important to extend initial work with vulnerability factors, that can more easily be obtained with rats, to a primate species since it is difficult to conduct these studies in humans. Another justification for using rhesus monkeys for the proposed experiments is that it allows for comparison of progress on this project with previously published work. There is an existing body of literature in which similar methods were used that is invaluable for

comparing results. Female monkeys are useful for investigating hormonal cycles because the length of their cycles and hormonal changes are more similar to humans than rats which cycle every few days. There are no computer simulations or other substitutes for examining the effects of drugs on behavior than intact, live, behaving nimals. Efforts have been made to minimize the number of animals studied in each experiment to the minimum needed to obtain statistically-significant effects. There are also plans to use within-subject designs in all experiments except those where sex or drug access history are the variables of interest.

3. Veterinary Care

Animal care is provided by the University Research Animal Resources staff, veterinarians, and veterinary technicians who make daily checks on the animals. Care is based on accepted and published principles of laboratory animal care (National Research Council, 1996). The veterinary staff is always available during working hours and on-call on holidays and weekends if any problems or illnesses occur. The veterinary staff takes blood samples for tests and monitors animals several times a day when illnesses occur. If minor surgery (e.g., abcessed tooth, hernia repair) is necessary they have the expertise in primate care to conduct the necessary procedures.

4. Procedures to minimize discomfort

There is no surgery or invasive procedures involved in these behavioral experiments. Drug injections will be given intramuscularly when medications are tested, and *blood samples will be collected by venapuncture for analysis of hormone and drug levels.* None of these procedures causes more than minor discomfort, and a small treat is usually given *to encourage the monkeys to extend their legs for a blood draw,* which encourages future cooperation. Twice yearly the monkeys are anesthetized with ketamine by the veterinary staff while TB tests are given and their teeth are cleaned.

5. Euthanasia

Monkeys are used sequentially in experiments; thus, they are not euthanized upon completion of an experiment. If older monkeys are replaced with younger monkeys, the older ones will be donated to another investigator. If euthanasia is required, Research Animal Resources *(RAR)* Veterinary Staff are contacted, and hey use an overdose of pentobarbital (100 mg/kg). *Prior to euthanasia they would perform various tests, (blood analysis, organ function, x-ray) in an attempt to diagnose and treat the illness. If the monkey does not improve after treatment,* criteria for euthanasia would be the inability to obtain food or water and/or unresponsiveness for a 24 hour period, *other criteria for euthanasia are unrepairable injury from the cage or two or three instances of self-injury. The RAR veterinarian* would then necropsy the animal to gain further information on the nature of the illness.

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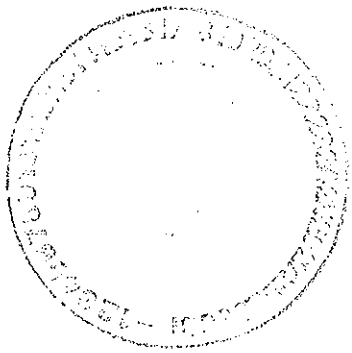
3E. Will you be conducting the same experiment in multiple species?

☐ Yes.
☒ No.

If yes, justify the need to do this:

Continue to Part B. If this experiment requires more than one species, complete a Part B for each species. Download an additional Part B of this form at *http://www.iacuc.umn.edu/download/*



Part B

If conducting an experiment with multiple species fill out Part B for each species. Download another copy of this part to fill out on the web at http://www.iacuc.umn.edu/download/

Identify the species this Part B addresses:

***Special instructions:** After printing this form, please write the species referred to in this part B on each page of part B in the space provided at the bottom before copying.*

4. Scientific Justification for Animal Species and Number Requested

4A. Justify the species to be used by indicating:

☐ **This is a new model.** (RAR veterinarians are available for consultation on new model development)

☒ **This model has previously been used. Provide citation and species:**

Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP (2004) Gender and hormones influence drug abuse. Trends in Pharmacoloogical Science, 25:273-279.

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☐ **The results will be directly applicable to the health, care or study of this species.**

☐ **This is agricultural research related to a particular farm animal.**

4B. Describe the features of the species (e.g., anatomic, physiologic, genetic, etc) that make it desirable for this model. Contrast with other available models, if any.

The data from drug abuse research in monkeys is very similar to data obtained from humans in laboratory experiments: however, the range of projects that can be accomplished in humans and the time participants are willing to be tested in the human laboratory or clinical research unit is very limited. Nonhuman primate research allows within subject comparisons under control and experimental conditions and control over the drug and behavioral history of the animal.

4C. How are the number of animals requested scientifically justified for this species (check all that apply and answer the subsequent questions):

☐ **Pilot study or preliminary project, group variances unknown at present. Explain justification for each species** (Minimal number of animals should be requested.):

☒ **Group sizes determined statistically. What statistical analysis was performed including the analysis employed and the power function?**

Previous statistical analyses and power calculations based on similar studies indicate that at least 7 animals per experiment are needed to statistical significance ($p<0.05$) in repeated measures factorial analyses of variance. Typically 7-9 animlas are used in each group for the experiments to account for one or two animals that may not have stable drug self-administration baselines.

☐ **Group sizes based on quantity of harvested cells or amount of tissue required. Explain how much tissue is needed based on the number of experiments you will conduct and how much tissue you expect to obtain from each animal:** (Suggestion: "The study requires 50 experiments." is not sufficient.)

☐ **Product Testing. If the number of animals needed is based on FDA guidelines, provide the citation from the regulations:**

☐ **Other - Elaborate, indicating criteria used to determine group size:**
(Suggestion: "This is the number used in the previous studies." is not sufficient. Statistical analyses should be available from prior studies.)

5. Potential Animal Pain and Distress

5A. What are the potential specific study-induced or related problems the animals might experience (i.e. health problems, pain, distress, complications, etc) OR any health problems due to the phenotype of the animal?

1. Describe the potential problems:

There may be minor discomfort associated with injections. On rate occasions, sedation or hyperactivity result from excessive drug self-administration, but this usually dissipates within 1 to 2 hours.

It is not expected that the animals will experience any withdrawal distress from the drugs that they are self-administering because the length of access to the drugs is short (1-2 hr), and withdrawal signs have not been noted with this length of access before.

2. Do you expect these problems to occur?

☒ **Yes.** Answer 2b-d.
☐ **No.** Answer 2a.

2a. Explain the basis for this assessment: (eg. prior experience, etc.)

2b. How will pain and/or distress be monitored? Provide the specific clinical signs which will be monitored as well as the frequency of monitoring, including provisions for off hours. Please note that animals housed outside of centrally managed facilities must be monitored daily.

The animals are observed several times each day, 7 days per week by lab personnel, and a vet tech or the attending vererinarian also monitor the animals regularly.

2c. Will this monitoring include weekends and holidays? (In addition to routine RAR monitoring)

☒ Yes.
☐ No.

2d. Explain what steps will be taken to alleviate any pain, distress or discomfort the animals may experience. Provide the dose, route of administration, frequency, and type of analgesic drugs or tranquilizers to be administered. (Suggestion: Consider warming pads, fluids, soft bedding, etc.)

If the monkeys are showing signs of intoxication, access to food is delayed by at least an hour or until the ataxia or hyperactivity subsides.

5B. Will cells, tissues, or body fluids be inoculated?

☐ Yes.
☒ No. Continue to section 6.

If yes, have they been screened for the presence of human or animal pathogens?

☐ Yes. Please provide documentation.
☐ No. RAR must be consulted to provide testing or to determine if there are special housing needs. Describe that consultation below:

6. Euthanasia/Disposition of Animals

6A. What will determine the natural endpoint(s) of the study?

(i.e., state the specific time points, state the specific tumor size, etc.)

When the aims of the specific experiments are accomplished

6B. Will the animals be euthanized at the end of the study?

☐ Yes.
☒ No.

If yes, specify method, agent and dosage and route of administration to be used for euthanasia for this species:
Euthanasia must be in accord with the methods approved by the AVMA Panel on Euthanasia. Note that the AVMA Panel does not allow cervical dislocation without anesthesia, unless scientifically justified. Please make sure to include the anesthetic regimen if proposing to use cervical dislocation. In addition, the AVMA panel on euthanasia does not allow dry ice as a source for carbon dioxide. If you choose to use carbon dioxide please confirm that you will use compressed carbon dioxide gas in cylinders.

If no, describe their final disposition:

6C. Will the animals be allowed to die as a result of experimental manipulation? (This means the animals are not euthanized.)

☐ Yes. Answer the question below.

☒ **No.** The time or criteria that determine that the experiment is over and animals are euthanized or removed from the study should be indicated in Question 3C. *It is understood that you will use Appendix N guidelines in addition to any of your own endpoints provided in question 3C.*

If yes, provide a scientific justification. If they are euthanized when they become "moribund", you must define or describe conditions that indicate moribundity. (Suggestion: Consider non-responsiveness to gentle prodding, loss of consciousness, etc.)

6D. Animals that are experiencing unrelieved pain or distress prior to the defined experimental endpoint must be humanely euthanized, unless doing so would interfere with, or compromise the scientific goals of the experiment. Do the guidelines interfere with your experimental objectives?

(Clinical signs of pain or distress that require euthanasia are listed in Appendix N)

☒ **No.** Initial and date that IACUC guidelines listed in Appendix N have been read and will be followed for early euthanasia.

MEG	10/20/04
PI Initials	**Date**

☐ **Yes.** Provide the criteria to be used by the PI to determine that euthanasia would be required prior to the end of the study AND provide scientific justification indicating why an earlier endpoint cannot be used:

6E. In the unexpected event that an animal meets these euthanasia criteria prior to the designated study endpoints, describe procedures to euthanize the animal: (provide agent, dosage, and route)

The monkey would be transferred to RAR facilities and be euthanized with an overdose of pentobarbital by RAR staff.

Reminder: *If conducting an experiment with multiple species fill out Part B for each species. Download another copy of PartB to fill out on the web at http://www.iacuc.umn.edu/download/*

You have reached the end of this form. Please make sure that you have responded to every question on this application (even if your response is "not applicable") and that you have filled out all of the applicable appendices.

Appendix C
Controlled Substances

Controlled Substances: Contact the Office of Regulatory Affairs at 612-625-9624 for controlled substances registration information or for guidance completing this section. Visit the web at http://www.dehs.umn.edu/ihsd/controlledsubs/ for more help.

Name of DEA registrant	Marilyn E. Carroll
Name of MNBP registrant	Marilyn E. Carroll
MNBP number (7 digits)	

Name of controlled drug or substance	DEA Drug Schedule (I, II, III)	Dosage	Do you use a controlled substance safe?	Do you maintain complete inventory and disposition use records?
Heroin	I	0.06, 0.12, 0.25, 0.5, 1.0 mg/kg	yes	yes
Cocaine	II	10 mg/kg	yes	yes
Methamphetamine	II	1 mg/kg	yes	yes
Phencyclidine	II	0.06, 0.12, 0.25, 0.5, 1.0 mg/ml	yes	yes
Pentobarbital	II	100 mg/kg	yes	yes
Methadone	II	0.8 mg/ml	yes	yes
Ketamine	III	10 mg/kg	yes	yes
Baclofen	IV	0.5, 1.25, 2.5 mg/kg	yes	yes
Bremazocine	IV	0.32, 1, 2.5 mg/kg	yes	yes