Notice of Research Fellowship Award

NATIONAL RESEARCH SERVICE AWARD **Federal Award Date:** 07/18/2017

Department of Heath and Human Services
National Institutes of Health

NATIONAL INSTITUTE ON DRUG ABUSE

Grant Number: 1F31DA043921-01 REVISED
FAIN: F31DA043921

Principal Investigator(s):
Kathryn Schwienteck, PHMD

Project Title: Immunopharmacotherapy for heroin addiction

Publow, Andrea J
Virginia Commonwealth University
800 East Leigh St, Suite 3200
PO Box 980568
Richmond, VA 232980568

Award e-mailed to: ospaward@vcu.edu

Period Of Performance:
Budget Period: 05/25/2017 – 05/24/2018
Project Period: 05/25/2017 – 05/24/2020

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $468 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to VIRGINIA COMMONWEALTH UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 288 42 CFR 66 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute On Drug Abuse of the National Institutes of Health under Award Number F31DA043921. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Pamela G. Fleming
Grants Management Officer
NATIONAL INSTITUTE ON DRUG ABUSE

Additional information follows

SECTION I – AWARD DATA – 1F31DA043921-01 REVISED

Award Calculation (U.S. Dollars)

Institutional Allowance	$4,200
Other	$7,738
Stipends	$23,844
Federal Direct Costs	$35,782
Total Award	$35,782
Total Amount of Federal Funds Obligated (Federal Share)	$35,782
TOTAL FEDERAL AWARD AMOUNT	$35,782
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$468

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$35,782	$35,782
2	$35,782	$35,782
3	$7,364	$7,364

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Drug Abuse and Addiction Research Programs
CFDA Number:	93.279
EIN:	1546001758A1
Document Number:	FDA043921A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017	2018	2019
DA	8472662	$35,782	$35,782	$7,364

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: MF/PAK / **OC:** 412L / **Released:** eRA Commons User Name 07/17/2017
Award Processed: 07/18/2017 00:03:04 AM

Fellow's e-mail:
Kathryn Schwienteck schwienteckl@vcu.edu

SECTION II – PAYMENT/HOTLINE INFORMATION – 1F31DA043921-01 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm.

SECTION III – TERMS AND CONDITIONS – 1F31DA043921-01 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) F31DA043921. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Fellowships that terminate early and awards in the final year are subject to NIH Closeout requirements. See Sections 8.6 and 11.2.11 of the NIH Grants Policy Statement Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final Federal Financial Report (FFR) (SF 425) on expenditures is not required for fellowships. However, submission of a final quarterly federal cash transaction report in the Payment Management System (PMS) is required if the fellowship funds are in a PMS P subaccount. A

final quarterly federal cash transaction report is not required for awards for fellowships (at Federal or foreign institutions) in PMS B subaccounts. NIH will close the fellowship using the last recorded cash drawdown level in PMS.

A Termination Notice is required in lieu of a final progress report. The termination notice must be submitted within 30 days of the termination date even if the fellow is not available for signature. In all cases, the information on the form must be verified by the sponsor and an institutional business official. The lack of timely and accurate information on this form could adversely affect data collected associated with aggregate NRSA support and the payback process. All Termination Notices for individual fellowships are required to be submitted electronically using the eRA Commons xTrain application.

An Activation Notice (PHS 416-5) must be submitted to the NIH awarding office as of the day the fellow begins training. Submission of a Payback Agreement form is also required for postdoctoral fellows in their first 12 months of Kirschstein-NRSA postdoctoral support. A payback agreement does not apply to predoctoral support. The applicable forms should be submitted to the awarding component at the following address:

National Institute on Drug Abuse
6001 Executive Blvd
Room 4237B, MSC 9550
Bethesda, MD 20892
Bethesda, MD 208929560

The Activation Notice and Payback Agreement forms are available at the following websites:
http://grants1.nih.gov/grants/funding/416/phs416-5.pdf and
http://grants1.nih.gov/grants/funding/416/phs6031.pdf

Fellows are required to notify the awarding unit as soon as they are aware of any possible change in plans regarding their fellowship support.

SECTION IV – DA Special Terms and Conditions – 1F31DA043921-01 REVISED

REVISION# _1_
This revised award increases the stipend level to the current level for FY2017, per the NIH Guide Notice for Grants and Contracts published on June 27, 2017.

NRSA PRE-DOCTORAL STIPENDS
This award reflects the stipend amount(s), tuition and fees, and institutional allowance amount(s) for Fiscal Year 2017 which are published in the June 27, 2017, NIH Guide for Grants and Contracts, at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-084.html.

TERMINATION NOTICE REMINDER
Should this fellowship terminate before the reflected budget/project period end date or a decision is made to not activate the fellowship, the sponsoring Recipient is to notify the NIDA Grants Management Specialist indicated on this Notice of Award (NoA) and submit a Termination Notice (PHS 416-7) immediately. Upon NIDA's receipt of the Termination Notice the NoA will be revised, accordingly. For instructions please see “8.5 Terminating Fellowships” found in the “eRA Commons xTrain External/Institutional User Guide.”

NIDA TERMS
In conjunction with the Acknowledgment of Federal Funding Requirement (as specified in the NIH Grants Policy Statement, Appropriation Mandates- http://grants.nih.gov/policy/nihgps/index.htm), in order to most effectively disseminate research results, advance notice should be given to NIDA that research finds are about to be published so that we may coordinate accurate and timely

release to the media. This information will be embargoed until the publication date. Any press notification should be coordinated with the NIDA Press Officer who can be reached at (301) 443-6245.

The National Institute on Drug Abuse (NIDA) encourages data harmonization to increase comparability, collaboration, and scientific yield of research on drug abuse. Towards that end, NIDA strongly encourages human-subject studies to incorporate a series of measures from the Substance Abuse and Addiction Core and Specialty collections, which are available in the PhenX Toolkit (www.phenxtoolkit.org>>>;;). For more information about NIDA's data harmonization efforts, please see NOT-DA-12-008 at http://grants.nih.gov/grants/guide/notice-files/NOT-DA-12-008.html.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Nadia Felix
Email: felixnl@mail.nih.gov **Phone**: 301-827-5701

Program Official: Philip A Krieter
Email: philip.krieter@nih.gov **Phone**: 301-435-7933

SPREADSHEET SUMMARY
GRANT NUMBER: 1F31DA043921-01 REVISED

INSTITUTION: VIRGINIA COMMONWEALTH UNIVERSITY

Budget	Year 1	Year 2	Year 3
Institutional Allowance	$4,200	$4,200	$2,100
Other	$7,738	$7,738	$1,290
Stipends	$23,844	$23,844	$3,974
TOTAL FEDERAL DC	$35,782	$35,782	$7,364
TOTAL FEDERAL F&A	$0	$0	$0
TOTAL COST	$35,782	$35,782	$7,364

PI: **Schwienteck, Kathryn**	Title: Immunopharmacotherapy for heroin addiction	
Received: 08/03/2016	FOA: PA16-309	Council: 01/2017
Competition ID: FORMS-D	FOA Title: RUTH L. KIRSCHSTEIN NATIONAL RESEARCH SERVICE AWARD (NRSA) INDIVIDUAL PREDOCTORAL FELLOWSHIP (PARENT F31)	
1 F31 DA043921-01	Dual:	Accession Number: 3959677
IPF: 353201	Organization: VIRGINIA COMMONWEALTH UNIVERSITY	
Former Number:	Department: Pharmacology and Toxicology	
IRG/SRG: ZRG1 F02A-K (20)L	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A)	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Sidney Negus PhD	Virginia Commonwealth University	Other (Specify)-Co-Sponsor
Justin Poklis	Virginia Commonwealth University	Other (Specify)-Significant Contributor
Matthew Banks	Virginia Commonwealth University	Other (Specify)-Sponsor
Kathryn Schwienteck	Virginia Commonwealth University	PD/PI

Reference Letters

Redacted by agreement

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*
❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED

Application Identifier
FP00001768

c. Previous Grants.gov Tracking Number
GRANT12224700

5. APPLICANT INFORMATION

Organizational DUNS*: 1053004460000

Legal Name*: Virginia Commonwealth University
Department: Pharmacology and Toxicology
Division:
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568

Person to be contacted on matters involving this application
Prefix: First Name*: Andrea Middle Name: J Last Name*: Publow Suffix:
Position/Title: Dir, OSP - Gov
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568
Phone Number*: 8048286772 Fax Number: 8048282521 Email: dirospa@vcu.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1546001758A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Immunopharmacotherapy for heroin addiction

12. PROPOSED PROJECT
Start Date* 04/01/2017
Ending Date* 05/31/2019

13. CONGRESSIONAL DISTRICTS OF APPLICANT
VA-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Kathryn Middle Name: L. Last Name*: Schwienteck Suffix:
Position/Title: Graduate Assistant
Organization Name*: Virginia Commonwealth University
Department: Pharmacology and Toxicology
Division:
Street1*: 410 N 12th Street
Street2: PO Box 980613
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980613
Phone Number*: 7178185890 Fax Number: Email*: schwienteckl@vcu.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$94,415.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$94,415.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Andrea Middle Name: J Last Name*: Publow Suffix:
Position/Title*: Dir, OSP - Gov
Organization Name*: Virginia Commonwealth University
Department:
Division:
Street1*: 800 East Leigh St, Suite 3200
Street2: PO Box 980568
City*: Richmond
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 232980568
Phone Number*: 8048286772 Fax Number: 8048282521 Email*: dirospa@vcu.edu

Signature of Authorized Representative*
Andrea.Publow

Date Signed*
08/03/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:160729_Cover_Letter.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Virginia Commonwealth University
Duns Number:	1053004460000
Street1*:	800 East Leigh St, Suite 3200
Street2:	PO Box 980568
City*:	Richmond
County:	
State*:	VA: Virginia
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	232980568
Project/Performance Site Congressional District*:	VA-003

File Name

Additional Location(s)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 02-10-2016

Animal Welfare Assurance Number A3281-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	160802_Project_Summary_KLS.pdf
8. Project Narrative*	160802_Project_Narrative_KLS.pdf
9. Bibliography & References Cited	160801_References_KLS.pdf
10.Facilities & Other Resources	FacilitiesResourcs_160731_KLS.pdf
11.Equipment	Equipment_160725_KLS.pdf
12. Other Attachments	Additional_Educational_Information.pdf

PROJECT SUMMARY

Heroin abuse and addiction is a major public health problem in the United States. Although there are FDA-approved heroin addiction pharmacotherapies, deaths from overdose are on the rise and current pharmacotherapies possess unwanted side effects. Immunopharmacotherapy has emerged as an alternative treatment option for heroin addiction and preclinical evaluation of a heroin vaccine immunopharmacotherapy represents a critical component in the addiction medication development process. This proposal will determine the efficacy and specificity of a heroin-TT conjugate vaccine in two behavioral procedures in nonhuman primates, while also assessing the impact the vaccine has on pharmacokinetic parameters of heroin. Aim 1 will determine the effects of the heroin-vaccine on abuse-related subjective effects in a two-key food-reinforced fentanyl vs. saline drug discrimination procedure in three males and three female rhesus monkeys. Heroin and its metabolites 6-acetylmorphine (6-AM) and morphine will be administered in a cumulative-dosing test procedure to determine their ability to produce fentanyl-like discriminative stimulus effects. Next a heroin-TT conjugate vaccine will be administered and dose-effect functions will be redetermined. Aim 2 will determine heroin-TT vaccine effects heroin and its metabolites antinociceptive properties in a warm-water tail withdrawal procedure. The same six monkeys will be trained to sit calmly in a chair, and tails will be dipped in warm water heated to 38°C, 50°C, and 54°C and baseline latencies to withdrawal tails from water will be determined. Test sessions will be conducted after administration of fentanyl, heroin, 6-AM, and morphine. Following baseline test determinations, the heroin-TT conjugate vaccine will be administered and the dose-effect curves will be redetermined. Aim 3 will correlate heroin pharmacokinetics with heroin behavioral effects before and after heroin-TT conjugate vaccine administration. Heroin, 6-AM and morphine levels will be analyzed and quantified by high-performance liquid chromatography–tandem mass spectrometry. Overall, the proposed research will improve our understanding of the potential utility of immunopharmacotherapy for the treatment of heroin addiction.

PROJECT NARRATIVE

Heroin addiction is a major public health crisis in the United States. Although there are FDA-approved pharmacotherapies for heroin addiction, current medications possess undesirable side effects. This application proposes preclinical research to evaluate a novel heroin-TT conjugate vaccine as a potential treatment option for heroin addiction in two behavioral procedures in male and female nonhuman primates.

BIBLIOGRPAHY & REFERENCES CITED

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FACILITIES AND OTHER RESOURCES

Laboratory Resources

The sponsor's laboratory is dedicated to research space for monkeys [Redacted by agreement] and is available for use by graduate students and laboratory technicians. All monkey lab space was recently renovated in 2007-2008 and is located within the AAALAC-accredited [Redacted by agreement]. Approximately [Redacted by agreement] of space will be dedicated to carrying out experiments in this proposal. There is sufficient space available for the proposed studies to be conducted for the entire funding period.

The three laboratory spaces that I will specifically use to conduct experiments include (1) a housing room equipped with 6 custom cages for monkeys involved in touchscreen operant studies, (2) an adjacent room that serves as a procedure room equipped with a surgical table and surgical light to be used for minor health-maintenance, blood-sampling or thermal nociception studies in chaired monkeys and (3) a "control" rooms that houses computer control equipment, administrative desk space for graduate students and technical staff, work space for drug preparation and equipment maintenance, and storage space for supplies. All rooms are temperature and humidity controlled and equipped with programmable lighting for controlled light-dark cycles. All housing rooms are equipped with automatic water-delivery systems.

Other general use resources are also available to me during the conduct of the proposed studies. These resources include the following: (1) steam and gas sterilizers for sterilization of supplies, (2) cage wash facilities for biweekly cage washing, and (3) walk-in refrigerators for storage of food and fruit. In addition, there is (4) a shop facility for design, manufacture and/or maintenance of equipment, and (5) a Mettler analytical balance and weighing table for use in weighing out drugs.

Animal Resources

The [Redacted by agreement] is accredited by the USDA and AAALAC, and it is operated by the Division of Animal Resources (DAR). The DAR is headed by three veterinarians [Redacted by agreement] [Redacted by agreement] with extensive nonhuman primate research experience. The veterinarians are supported by two veterinary technicians who also have experience with nonhuman primate medicine and research. All animals are checked daily by the DAR staff as well as by our own technical staff, and DAR staff are also available to assist as necessary with diagnosis and treatment of any health issues. A large animal care staff under DAR direction performs daily husbandry and assists as necessary with feeding, importation and quarantine of new animals, and maintenance of animal facility infrastructure. Physical resources available through DAR include housing space and cages, food-storage refrigerators, cage washing equipment, and a surgical suite for aseptic surgeries in rhesus monkeys.

Behavioral equipment

Six touchscreen monitors and two backup monitors are available to be attached to the front of the monkey's cage during behavioral tasks. IBM-compatible computers and associated interface equipment (ABET II Interface and Software from Lafayette Instrument ®, and Whisker Control by Cambridge University Technical Services, Ltd) are used to provide input and receive output from the touchscreen stations. A total of 2 PC computers are dedicated to the six monkey operant stations. An additional computer and touchscreen monitor is available as a backup and is used as part of a test station for program development and equipment diagnostics. All experimental computers are connected to surge and battery backup devices that are plugged into emergency generator supported electrical circuits. All software is custom written.

Office Resources

Shared office space is available for graduate students in Dr. Bank's laboratory. In addition, other departmental and institutional office resources are also available for the proposed research. The Pharmacology and Toxicology business office is located on the same floor and is responsible for carrying out and placing supply orders associated with my grant. Physical resources including a scanner, fax machine and copier are also available. All graduate students have access to apple computers for data processing, word processing, and administrative and regulatory responsibilities. These computers contain: GraphPad, MS Office, EndNote and others.

Department of Pharmacology Facilities

There is a departmental mass spectrometry core directed full-time by Mr. Justin Poklis and the departmental machine shop (McGuire Hall) is operated by [Redacted by agreement]

Other Resources

Significant other resources include the following:

1) Equipment for preparation of drug solutions, including a sonicator, stirrer, and Mettler Analytical balance
2) An inventory of tools and spare parts for equipment maintenance
3) A DEA approved safe for storage of schedule I-V drugs
4) A centrifuge for spinning blood samples and isolating serum
5) Access to a -80°F freezer for storage of serum samples
6) Access to an isoflurane gas anesthesia machine for health examinations
7) Six restraint chairs for chair monkeys

EQUIPMENT

The monkey laboratory is equipped with 6 custom cages (Redacted by agreement). All cages have custom fronts designed to accommodate attachment of touch sensitive operant panels. Cages are also equipped with perches and squeeze backs, and they are mounted on racks equipped with tubing for automatic water delivery to each cage. The cages are designed to chair monkeys using the pole and collar technique.

Each operant cage is equipped with a detachable, custom-built touch sensitive operant panel. Operant panels are controlled by computers and interface equipment described above under "Behavioral Equipment." Each cage is equipped with a pellet dispenser (ENV-203-1000; Med Associates), and custom programs will be written in ABET-II notation. The entire touchscreen station is plugged into a dedicated UPS surge protector and battery backup device.

Six restraint chairs, along with stainless steel pole and collars, are available for chairing monkeys.

ADDITIONAL EDUCATIONAL INFORMATION

PhD students in Pharmacology and Toxicology take courses designed for graduate students with an emphasis on research design and experimentation. A full-time course load for graduate students is 15 credits in the fall and spring semesters and six credits in the summer. Students joining the Department of Pharmacology and Toxicology are required to complete the following Pharmacology courses:

1) PHTX 630 – Basic Concepts in Pharmacology (3 credit hours) offered in the Spring Semester of the first year
2) PHTX 536 – Principles of Pharmacology (5 credit hours) – Fall semester (2^{nd} year)

Since Ms. Katie Schwienteck has a PharmD degree, PHTX 536 is not required. She will take additional advanced electives pertinent to her project.

In addition to the mandatory Pharmacology courses, students are encouraged to take Biochemistry (5 credit hrs: 503) and Microbiology/Immunology (5 credit hrs: 504) and/or other advanced electives prior to taking the qualifying examinations. The advanced electives in Pharmacology vary from 1-5 credits and include courses that include Neuropharmacology, Behavioral Pharmacology, Introduction to Toxicology, Cellular Signaling, etc.

Student coursework and progress is monitored by the Graduate Program Director and evaluation of student progress is reported to the Departmental faculty on a semi-annual basis. At the end of the first year each student together with their mentor is required to appoint a student advisory committee that consists of 5 faculty members (3 from Pharmacology and Toxicology and 2 from outside the Department). The advisory committee is required to meet with the student at least twice a year, increasing in frequency as the student research progresses. Advisory committee reports are prepared by the committee and submitted to the Graduate Program Director at the end of each meeting.

The Department of Pharmacology and Toxicology at Virginia Commonwealth University has a rich history of graduate education with more than 310 PhDs graduated over the last 50 years. Over the last 10 years, 75 PhD degrees in Pharmacology and Toxicology have been awarded by the Department and the average time to degree has been 4.8 years.

Progress/Status of Kathryn L. Schwienteck

Katie has a current GPA Personal Info Kathryn has completed all core course requirements for the PhD program in Pharmacology and advanced electives in Biochemistry (BIOC 530), Behavioral Pharmacology (PHTX 633), and will be taking the additional class in Pain Pharmacology (PHYX 691). Katie is also currently enrolled in responsible scientific conduct (OVPR602). Katie's project is to determine the efficacy of heroin vaccine on abuse-related effects, antinociception and to correlate these with pharmacokinetics. Kathryn joined our program after completion of a PharmD, is hard working and a highly motivated individual who plans to develop into an independent scientist.

Hamid I. Akbarali,
PhD Graduate Program Director,
Department of Pharmacology and Toxicology
Virginia Commonwealth University

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Degree Type: PharmD Degree Year: 2015

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Attach Current & Pending Support:	

PROFILE - Senior/Key Person

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PROFILE - Senior/Key Person

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Degree Type: PhD Degree Year: 1990

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Attach Current & Pending Support:

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Significant Contributor

Degree Type: BS Degree Year: 1996

File Name
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Attach Current & Pending Support:

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME OF APPLICANT: Kathryn Schwienteck

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Graduate Student Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
University of Pittsburgh, Pittsburgh, PA	B.S	08/2007	05/2011	Neuroscience
Virginia Commonwealth University, Richmond, VA	PharmD.	08/2011	05/2015	Pharmacy
Virginia Commonwealth University, Richmond, VA	Ph.D.	08/2015	In Progress	Pharmacology and Toxicology

A. Personal Statement

My career aspiration is to become an independent drug abuse researcher and this fellowship will help me to achieve that goal. I believe that I am a uniquely qualified candidate for this F31 fellowship due to my clinical background in pharmacy in treatment human diseases, my previous research experiences and my present curiosity and interest in the study of how drugs produce their addictive effects. As a pharmacist, my clinical experience and knowledge will provide me with a unique skillset and perspective to contribute to basic science research in drug abuse. While in pharmacy school, I volunteered for many hours at health clinics, and spent time with patients who were often afflicted by substance-use disorders. Furthermore, I saw first-hand patients who had overdosed on heroin during a rotation at the Virginia Poison Center and am acutely aware of the adverse health consequences of drug abuse. Moreover, I feel confident to lead and carry out the proposed research because my academic and laboratory environment within the Department of Pharmacology and Toxicology at VCU. During my undergraduate studies, I was first exposed to research while working as an undergraduate assistant in Dr. Charles Bradberry's nonhuman primate laboratory at the University of Pittsburgh. As a pharmacy student at Virginia Commonwealth University, I conducted research with Dr. Matthew Banks on the effects of candidate medications on methamphetamine self-administration in monkeys, and the effects of subchronic nicotine treatment and withdrawal on cocaine self-administration in monkeys. These two experiments resulted in two co-authored manuscripts with Dr. Banks and also provided me with the opportunity to present the results at a VCU School of Pharmacy Research and Career day in poster format in which my poster presentation won "Best PharmD Poster Award." In my first year of graduate school, I had the opportunity to conduct acute and chronic drug studies in an intracranial self-stimulation (ICSS) procedure in rodents with Dr. Steve Negus and collaborators from the University of Wisconsin. I presented results from these experiments at three conferences in poster format, including the 2016 Behavior, Biology, and Chemistry: Translational Research in Addiction conference in San Antonio, in which I applied for and received a travel stipend to attend. I possess a robust set of skills, training, education and experience necessary to complete the proposed research project, however, this project will introduce me to new areas of research (e.g. immunopharmacotherapy, drug discrimination, and preclinical models of acute pain) while also teach me new techniques including programming, pharmacokinetic analysis, and new behavioral procedures. Overall, this proposal will facilitate my graduate education in experimental methods related to assessment of potential medication treatments for substance use disorders, sharpen my writing, informal and formal data presentation skills as well. Overall, the training and mentorship I will receive during this fellowship will provide a strong foundation upon which to pursue my next career step of becoming an independent researcher.

1. Banks ML, Hutsell BA**, Schwienteck KL,** Negus SS. Use of preclinical drug vs. food choice procedures to evaluate candidate medications for cocaine addiction. Current Treatment Options in Psychiatry: 2015; 2(2): 136-150, PMC4441409

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE MM/YYYY	END DATE MM/YYYY	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate research technician	09/2009	12/2010	Neuroscience	University of Pittsburgh	C.W. Bradberry
Pharmacy Student	08/2011	05/2015	Pharmacy	Virginia Commonwealth University	V. Yanchick/J.T. Dipiro
Research Assistant	01/2015	08/2015	Pharmacology	Virginia Commonwealth University	M.L. Banks/S.N. Negus
Pharmacist	05/2015	Present	Pharmacy		
Graduate Student Research Assistant	08/2015	Present	Pharmacology	Virginia Commonwealth University	M.L. Banks

Professional Memberships

(2016-Present) Virginia Academy of Science (VAS), *Member*

Honors and Awards

2011 Bachelor of Science, Cum Laude – University of Pittsburgh
2011 VCU Student National Pharmaceutical Association Member of the Month
2012 VCU Student National Pharmaceutical Association Member of the Year
2012 Phi Lambda Sigma Leadership Recognition Award
2013 Distinguished Service Award– Student National Pharmaceutical Association
2013 A.D. Williams and School of Pharmacy Summer Research Fellowship
2013 Research Day PharmD Student Award - VCU School of Pharmacy Research and Career Day
2015 Doctorate of Pharmacy, Cum Laude – VCU School of Pharmacy
2016 Behavior, Biology, and Chemistry: Translational Research in Addiction travel stipend award

C. Contributions to Science

1. Pharmacy School Research:
   I. During the summer in between my second and third year of pharmacy school, I applied for and received a fellowship to conduct research in the laboratory of Dr. Matthew Banks. During my time in the laboratory, I worked on a project that studied the effects of subchronic nicotine exposure and withdrawal from nicotine on the reinforcing effects of cocaine self-administration in rhesus monkeys. This experiment was my first exposure to the field of behavioral pharmacology and I learned the advantages of using a drug versus food choice procedure to model drug-taking behavior in laboratory animals. I worked directly with Dr. Banks to establish the experimental design of the study. During the fellowship, I carried out experiments which included daily upkeep of experimental equipment, recording of data, monitoring monkey's health and catheter status throughout the study, and observing for signs and symptoms of withdrawal from nicotine treatment. After the experiment, I learned how to analyze and present the data, wrote the first draft of the manuscript and assisted with edits after the peer review process. The work from this project culminated in a poster presentation and manuscript.
      a. **Schwienteck KL**, Negus SS, Poklis JL, Banks, ML. Effects of continuous nicotine treatment and subsequent termination on cocaine versus food choice in male rhesus monkeys. Exp Clin Psychopharmacol: 2015; 23(5): 395-404, PMC4579004
      b. **Schwienteck KL**, Banks ML. Effects of chronic nicotine treatment and nicotine withdrawal on choice between cocaine and food in rhesus monkeys. Poster presented at: VCU School of Pharmacy Research and Career Day; 2013 Nov 8; Richmond, VA
   II. During my final year of pharmacy school, I rejoined Dr. Bank's laboratory and worked on a project that studied the effects of d-amphetamine, methylphenidate, and cocaine treatment on the reinforcing

effects of methamphetamine choice in a self-administration procedure in monkeys. I carried out daily experiments which included decided when treatments should be initiated based on monkey's baseline behavior, monitored monkey's health throughout the experiments and tended to daily equipment upkeep. This experiment helped solidify my understanding of choice experiments, and I learned how monkeys are trained up to respond in a choice procedure, as methamphetamine is a particularly challenging drug to establish as a reinforcer in choice procedures. Results from this experiment culminated in a manuscript, which I wrote and assisted in the revisions post-peer review. I also presented data from this project at a joint lab exchange at Emory University.

a. **Schwienteck KL**, Banks ML. Effects of 7-day continuous d-amphetamine, methylphenidate, and cocaine treatment on choice between methamphetamine and food in male rhesus monkeys. Drug Alcohol Depend: 2015; 155:16-23, PMC4582002
b. **Schwienteck KL**, Banks ML. Effects of candidate agonist-based medications on methamphetamine vs. food choice in monkeys. Oral presentation at: WFU/VCU/Emory Annual Lab Exchange; 2015 Aug; Atlanta, GA.

2. Graduate Research:

I. During my rotation in Steve Negus's lab, I learned how to conduct a new behavioral procedure, intracranial self-stimulation, which studies motivated behavior in rodents. I learned how to surgically implant an electrode in a rat brain and how to train a rat on a frequency-rate program to respond on a lever for brain stimulation. My first project in his laboratory was part of a collaborative experiment with several other laboratories at VCU. I tested 4 different synthetic cathinone-analogs on the effects of ICSS. The cathinone-analogs increased in side-chain length and the general premise was that as the length of the side chain increased, the compounds would produce more rate-decreasing effects instead of rate-increasing effects on ICSS. Results confirmed the hypothesis and a manuscript is currently being prepared.

a. Sakloth F, Eltit JM, Solis, Jr. E, Partilla JS, Ruchala I, **Schwienteck K**, Baumann MH, De Felice LJ, Glennon RA, Negus SS. Integrated strategy for drug discovery with monoamine transporter ligands: application to structure-activity studies with 4-methylamphetamine analogs. Poster presented at: Virginia Drug Discovery Consortium, Virginia BrainRx: A Symposium on Drug Discovery for the Brain; 2016 May 23-24; Richmond, VA.

II. My second project in Dr. Negus's laboratory was to examine the effects of $GABA_A$ positive allosteric modulators (PAMs) on ICSS in rodents. I performed surgery on all rats for this surgery and trained them to respond under a frequency-rate program for brain stimulation. During this project I studied five different compounds that varied for their selectivity at different $GABA_A$ subunits. We learned that nonselective compounds such as diazepam and alpha-1 selective compounds, zolpidem will produce rate-increasing effects in ICSS, which we predict contributes to their abuse liability. Compounds that are selective for alpha-2/alpha-3 subunits do not produce rate-increasing effects in ICSS. After acute drug studies, I conducted a 25 consecutive day-long experiment that examined the effects of subchronic diazepam administration on cumulative dosing of diazepam. Results from this study show that subchronic diazepam treatment produced tolerance to the rate-decreasing effects of diazepam but did not unmask any rate-increasing effects. I have presented data from this project at three different conferences and will be preparing the manuscript at the end of this summer.

a. **Schwienteck, KL**, Li, G, Poe, MM, Cook, JM, Banks, ML, & Negus, SS. *Abuse-related effects of $GABA_A$ receptor positive allosteric modulators in an assay of intracranial self-stimulation in rats*. Poster presented at: Behavior, Biology, and Chemistry: Translational Research in Addiction; 2016 Mar 5-6; San Antonio, TX.

URL of publications: http://www.ncbi.nlm.nih.gov/pubmed/?term=schwienteck

D. Additional Information: Research Support and/or Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE
	University of Pittsburgh	
2007	General Chemistry I	Personal Info
2007	Drugs and Behavior	
2008	Foundations of Biology I	
2008	General Chemistry II	

YEAR	OTHER COURSE TITLE	GRADE
	University of Pittsburgh	
2007	Mesoamerica Before Cortez	Personal Info
2007	Introduction to Psychology	
2007	Introduction to the Arts & Sciences	
2008	Health Focus	

YEAR	SCIENCE COURSE TITLE	GRADE
2008	Foundations of Biology Lab 1	Personal Info
2008	Foundations of Biology 2	
2008	Organic Chemistry 1	
2008	Organic Chemistry Laboratory 1	
2009	Foundations of Biology Lab 2	
2008	Organic Chemistry 2	
2009	Organic Chemistry Laboratory 2	
2009	Introduction to Neuroscience	
2009	Functional Neuroanatomy	
2009	Independent Study – Research	
2009	Introduction to Physics 1	
2010	Synaptic Transmission	
2010	Introduction to Physics 2	
2010	Independent Study – Research	
2010	Introduction to Laboratory Physics	
2010	Biochemistry	
2010	Independent Study – Research	
2010	Neurochemical Basis of Behavior	
2010	Human Physiology	
2011	Neurophysiology	
2011	Psychiatric Disorders & Brain Function	
	Community College of Allegheny County	
2011	Microbiology	
2011	Anatomy & Physiology II	
	Virginia Commonwealth University School of Pharmacy	
2011	Pharmacognosy	
2011	Pharmaceutics & Biopharmaceutics I	
2012	Clinical Chemistry for the Pharmacist	
2012	Clinical Therapeutics Module I: Introduction to Medicinal Chemistry	
2012	Pharmacokinetics	
2012	Pharmaceutics & Biopharmaceutics II	
2012	Clinical Therapeutics Module II: Introduction to Pharmacology	
2012	Clinical Therapeutics Module III: Introduction to Special Populations	
2012	Clinical Therapeutics Module IV: Cardiovascular	

YEAR	OTHER COURSE TITLE	GRADE
2008	Seminar in Composition	Personal Info
2008	Trigonometry & Functions	
2008	Analytic Geometry and Calculus 1	
2008	Origins of Christianity	
2009	Introduction to World Art	
2009	Magic Medicine and Science	
2009	Reading Poetry	
2009	Renaissance Art	
2009	Pilates	
2010	Introduction to Macroeconomic Theory	
2010	Personal Fitness	
2010	Public Speaking	
2010	History of Ancient Philosophy	
2011	Introduction to Microeconomic Theory	
2011	Sensation and Perception	
2011	Basic Applied Statistics	
2011	Neuroscience/Writing Practicum	
	Universität Heidelberg	
2009	German I	
	Community College of Allegheny County	
2011	Contemporary History of U.S.	
	Virginia Commonwealth University School of Pharmacy	
2011	Basic pharmaceutical principles for pharmacist	
2011	Evidence-Based Pharmacy I: Introduction to Pharmacy Skills	
2011	U.S. Health Care System	
2011	Managing Professional Patient-Centered Practice	
2011	Foundations I	
2012	Contemporary Pharmacy Practice	
2012	Foundations II	
2012	Self-Care and Alternative and Complementary Treatment	
2012	Foundations III	

YEAR	SCIENCE COURSE TITLE	GRADE
2012	Clinical Therapeutics Module V: Endocrinology	Personal Info
2012	Clinical Therapeutics Module VI: Neurology I	
2013	Applied Pharmacokinetics	
2013	Clinical Therapeutics Module VII: Neurology II	
2013	Clinical Therapeutics Module VIII: Psychiatry	
2013	Clinical Therapeutics Module IX: Respiratory & Immunology	
2013	Clinical Therapeutics Module X: Infectious Disease	
2013	Clinical Therapeutics Module XI : Hematology & Oncology	
2013	Clinical Therapeutics Module XII: Nephrology & Urology	
2013	Drug Dependence	
2013	Clinical Therapeutics Module XIII: Dermatology & EENT	
2014	Clinical Therapeutics Module XIV: Gastrointestinal/Nutrition	
2014	Clinical Therapeutics Module XV: Women's Health/Bone and Joint	
2014	Clinical Therapeutics Module XVI: Critical Care	
2014	Special Topics: Veterinary Medicine	
2014	Clinical Therapeutics Module XVII: Dermatology & EENT	
2015	Clinical Therapeutics Module XVIII: Special Populations	
	Virginia Commonwealth University (Graduate Courses)	
2015	Directed Research in Pharmacology	
2015	Directed Research in Pharmacology	
2015	Pharmacology Research Seminar	
2015	Biochemistry Module 1: Protein Structure & Function	
2015	Special topics in Interdisciplinary Biomedical Sciences	
2016	Basic Concepts in Pharmacology	
2016	Behavioral Pharmacology	
2016	Pharmacology Research Seminar	
2016	Directed Research in Pharmacology	

YEAR	OTHER COURSE TITLE	GRADE
2012	Evidenced-Based Pharmacy II: Research Methods and Statistics	Personal Info
2012	Evidenced-Based Pharmacy III: Drug Literature Evaluation	
2012	Pharmacy Informatics	
2013	Foundations IV	
2013	Pharmacoeconomics	
2013	Epidemiology & Pharmacy Practice	
2013	Patient Medication Safety	
2013	Foundations V	
2014	Foundations VI	
2014	Pharmacy Law	
	Virginia Commonwealth University (Graduate Courses)	
2015	Laboratory Safety	Personal Info

S indicates Satisfactory, W indicates Withdrawn before end of term.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Matthew L. Banks

eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ohio Northern University, Ada, OH	PharmD	1997-2003	Pharmacy
Wake Forest University, Winston-Salem, NC	PhD	2003-2007	Physiology and Pharmacology
Yerkes National Primate Research Center, Emory University, Atlanta, GA (Postdoctoral)		7/2007-6/2008	Behavioral Neuroscience
Virginia Commonwealth University, Richmond, VA (Postdoctoral)		7/2008-6/2010	Behavioral Neuroscience

A. Personal Statement

I am a trained pharmacist and behavioral pharmacologist who has been investigating the neurochemical and behavioral effects of abused drugs my entire research career. This combined clinical and basic science background has been advantageous in the experimental design development of preclinical studies related to understanding the neurobiological and environmental mechanisms of drug addiction. Moreover, as summarized below and demonstrated in Section C. Contributions to Science and Section D. Research Support, my +10 years of experience with conducting sophisticated behavioral procedures in nonhuman primates qualifies me to serve as the sponsor on this predoctoral NRSA F31 fellowship application titled "Immunophamacotherapy for heroin addiction". I have a strong commitment and desire to train the next generation of drug abuse researchers. This commitment has been empirically demonstrated by the participation of laboratory technicians and summer undergraduate students in the peer-reviewed manuscript process as highlighted below. Since 2003, my research program has utilized rhesus monkeys, the proposed research subjects in this application. Thus, from both a conceptual and technical standpoint, I am extremely qualified to serve as the sponsor on this predoctoral F31 application.

1) **Banks ML**, Haynes K, Sprague JE (2009) A model for motivating PharmD students to pursue a PhD. *Currents in Pharmacy Teaching and Learning* 1:93-97.
2) **Banks ML**, Gould RW, Czoty PW, Nader MA (2008) Relationship between response rates and measures of reinforcing strength using a choice procedure in monkeys. *Behav Pharmacol* 19:365-9. PMC2705207
3) Schwienteck KL, Negus SS, Poklis JL, **Banks ML** (2015) Effects of continuous nicotine treatment and subsequent termination on cocaine vs. food choice in male rhesus monkeys. *Exp Clin Psychopharmacol* 23:395-404. PMC4579004.
4) Smith DA, Negus SS, Poklis JL, Blough BE, **Banks ML** (2016) Cocaine-like discriminative stimulus effects of alpha-pyrrolidinovalerophenone, methcathinone, and their 3,4-methylenedioxy or 4-methyl analogs in rhesus monkeys. *Addict Biol*, doi: 10.1111/adb.12399. PMC – In Progress.

B. Positions and Honors

Positions and Employment

11/2006 – 5/2007	Instructor, Division of Nursing, Winston Salem State University
10/2007 – 10/2008	Adjunct Faculty, Dept. of Biomedical Sciences, Ohio Northern University
7/2010 – 1/2015	Research Assistant Professor, Dept. Pharmacology & Toxicology, Virginia Commonwealth University
1/2013 – Present	Faculty, Institute for Drug and Alcohol Abuse Studies, Virginia Commonwealth University
7/2015 – Present	Tenure-Track Assistant Professor, Dept. Pharmacology & Toxicology, Virginia Commonwealth University

Academic and Professional Honors

2002	Charles Oren Lee Research Award
2003	DeBow Freed Outstanding Senior Male Leadership Award
2004-2005	Wake Forest University NIDA Training grant appointee (Predoctoral)
2006	Society for Neuroscience Chapters Graduate Student Travel Award
2006	Young Investigator Travel Award, Frontiers in Addiction Research
2006	Biomedical Sciences Alumni Student Travel Award
2007	David K. Sundberg Award
2007	Western North Carolina Society for Neuroscience Chapter Poster Competition Finalist
2007-2008	Emory University NIDA Training grant appointee (Postdoctoral)
2008	ASPET Young Investigator Travel Award
2008-2010	Virginia Commonwealth University NIDA Training grant appointee (Postdoctoral)
2009	College on Problems of Drug Dependence Early Career Investigator Travel Award
2010	NIDA Director's Travel Award for CPDD Annual Meeting
2013	American College of Neuropsychopharmacology Travel Awardee
2016	Outstanding Early Career Faculty Award, Virginia Commonwealth University

Professional Societies

2008 – Present	Member, American Society of Pharmacology and Experimental Therapeutics
2008 – Present	Member, Behavioral Pharmacology Society
2014 – Present	Member, College on Problems of Drug Dependence
2015 – Present	Associate Member, American College of Neuropsychopharmacology

C. Contribution to Science: In total, I have authored over 69 peer-reviewed publications and five book chapters

1. I also have a long-standing interest in correlating plasma drug levels and other pharmacological dependent measures. This interest serves as a core foundation for the proposed studies in this predoctoral fellowship application. We have demonstrated the role of phenmetrazine as an active metabolite of phendimetrazine in contributing to phendimetrazine's abuse-related effects in a cocaine discrimination procedure and for phendimetrazine's therapeutic effects in attenuating cocaine vs. food choice. In addition, we have recently extended these findings to the prodrug formulation lisdexamfetamine. Furthermore, we have demonstrated that methamphetamine metabolism to amphetamine is not a primary mediator of methamphetamine's discriminative stimulus effects.

    1) **Banks ML**, Blough BE, Fennell TR, Snyder RW, Negus SS (2013) Role of phenmetrazine as an active metabolite of phendimetrazine: Evidence from studies of drug discrimination and pharmacokinetics in rhesus monkeys. *Drug Alcohol Depend* 130:158-166. PMC361650
    2) **Banks ML**, Hutsell BA, Blough BE, Poklis JL, Negus SS (2015) Preclinical assessment of lisdexamfetamine as a candidate "agonist" medication for cocaine addiction: Effects in rhesus monkeys trained to discriminate cocaine or to self-administer cocaine in a cocaine-vs.-food choice procedure. *Int J Neuropsychopharmacol*, doi: 10.1093/injp/pyv009. PMC4458439
    3) **Banks ML**, Smith DA, Kisor DF, Poklis JL (2015) Relationship between discriminative stimulus effects and plasma methamphetamine and amphetamine levels of intramuscular methamphetamine in male rhesus monkeys. *Pharmacol Biochem Behav* 141:58-65. PMC4724286
    4) Negus SS, **Banks ML** (2016) *Pharmacokinetic-Pharmacodynamic (PKPD) Analysis with Drug Discrimination in Rhesus Monkeys*. In: Porter JH, Prus AJ, editors. The Behavioral Neuroscience of Drug Discrimination, Springer, New York, NY.

2. Another core component of my research program has been the use of drug discrimination procedures to understand the behavioral pharmacological mechanisms of the target abused drugs subjective-like effects in both nonhuman primate and rodent drug discrimination procedures. These results have yielded three main findings. First, we have reported species differences in the substitution profile of nicotinic acetylcholine receptor agonists and antagonists for the discriminative stimulus effects of cocaine and methamphetamine. Second, we have reported species differences in the time course of prodrugs, such as phendimetrazine, to produce cocaine-like discriminative stimulus effects. Lastly, we have recently demonstrated that nicotinic acetylcholine receptor antagonism does not contribute to the "agonist-like" properties of bupropion in a methamphetamine drug discrimination procedure. I have served as either the Principal Investigator or Co-Investigator on grants associated with the published primary research listed below.

    1) **Banks ML** (2014) Effects of the nicotinic acetylcholine receptor antagonist mecamylamine on the discriminative stimulus effects of cocaine in male rhesus monkeys. *Exp Clin Psychopharmacol*. PMC40467453
    2) **Banks ML**, Bauer CT, Blough BE, Rothman RB, Partilla JS, Baumann MH, Negus SS (2014) Abuse-related effects of dual dopamine/serotonin releasers with varying potency to release norepinephrine in male rats and rhesus monkeys. *Exp Clin Psychopharmacol* 22:274-284. PMC4067459
    3) Bauer CT, Negus SS, Blough BE, **Banks ML** (2016) Cocaine-like discriminative stimulus effects of phendimetrazine and phenmetrazine in rats. *Behav Pharmacol* 27 (2 and 3 – Special Issue):192-195. PMC4779707
    4) **Banks ML**, Smith DA, Blough BE (2016) Methamphetamine-like discriminative stimulus effects of bupropion and its two hydroxyl metabolites in male rhesus monkeys. *Behav Pharmacol* 27 (2 and 3 – Special Issue):196-203. PMC4779668

3. Another focus of my current research program has been the development of adjuncts that enhance the therapeutic-like effects of mu-opioid receptor agonists, such as antinociception, and attenuate the undesirable effects of mu-opioid receptor agonists, such as sedation. The results of this research program have demonstrated 2 main findings. First, we have found that delta-opioid receptor agonists produce the desired therapeutic profile of enhancing mu-opioid receptor agonists without also enhancing suppression of operant response rates. Moreover, we have reported that mu-opioid agonists, such as morphine, fentanyl, and methadone may have delta-opioid agonist activity by activating mu-delta heterodimers. Second, we have extended these findings to the serotonergic system, suggesting that serotonin uptake inhibitors may also have clinical utility as opioid adjuncts and that lower efficacy mu-opioid receptor agonists are more amendable to serotonergic modulation. I have been a Co-Investigator on grants associated with the published primary research listed below and am a co-Principal Investigator on a grant to determine the utility of NMDA antagonists as opioid adjuncts.

    1) **Banks ML**, Folk JE, Rice KC, Negus SS (2010) Selective enhancement of fentanyl-induced antinociception by the delta agonist SNC162 but not ketamine in rhesus monkeys: further evidence supportive of delta agonists as candidate adjuncts to mu opioid agonists. *Pharmacol Biochem Behav* 97:205-212. PMC2967222
    2) **Banks ML**, Rice KC, Negus SS (2010) Antinociceptive interactions between mu-opioid agonists and the serotonin uptake inhibitor clomipramine in rhesus monkeys: role of mu agonist efficacy. *J Pharmacol Exp Ther* 335:642-650. PMC2967402
    3) Aceto MD, Harris LS, Negus SS, **Banks ML**, Hughes LD, Akgun E, Portoghese PS (2012) MDAN-21: A bivalent opioid ligand containing mu-agonist and delta-antagonist pharmacophores and its effects in rhesus monkeys. *Int J Med Chem* 2012, Article ID 327257, doi:10.1155/2012/327257.
    4) Yekkirala AS*, **Banks ML***, Lunzer MM, Negus SS, Rice KC, Portoghese PS (2012) Clinically employed opioid analgesics produce antinociception via mu-delta opioid receptor heterodimers in rhesus monkeys. *ACS Chem Neurosci* 3:720-7. doi:10.1021/cn300049m. *denotes equal contribution. PMC3447399

4. Another focus of my research program has been the extension of drug vs. food choice to other abused drugs. In particular, we have recently developed a methamphetamine vs. food choice procedure in rhesus

monkeys. The results have led to two main findings. First, monoamine uptake inhibitor treatment and dopamine antagonist treatment do not significantly attenuate methamphetamine vs. food choice. Second, treatment with the monoamine releaser amphetamine did not significantly decrease methamphetamine choice, but did decrease methamphetamine choice in two out of four monkeys. These later results suggest individual differences in treatment sensitivity and further highlight that different treatment strategies are necessary for different abused drugs. I have been the Primary Investigator on grants associated with the published primary research listed below.

1) **Banks ML**, Blough BE (2015) Effects of environmental manipulations and treatment with bupropion and risperidone on choice between methamphetamine and food in rhesus monkeys. *Neuropsychopharmacology* 40:2198-2206. PMC4613609
2) Schwienteck KL, **Banks ML** (2015) Effects of 7-day continuous d-amphetamine, methylphenidate, and cocaine treatment on choice between methamphetamine and food in rhesus monkeys. *Drug Alcohol Depend* 155:16-23. PMC4582002
3) **Banks ML** (2016) Effects of 7-day repeated 5-HT_{2A} inverse agonist/antagonist pimavanserin treatment on choice between methamphetamine and food in male rhesus monkeys. *Drug Alcohol Depend,* 165:260-264. doi: 10.1016/j/drugalcdep.2016.05.014. PMC4939103.

Complete List of Published Work:
http://www.ncbi.nlm.nih.gov/sites/myncbi/matthew.banks.2/bibliography/44032664/public/?sort=date&direction=ascending

D. Research Support

Ongoing:

R01DA026946 (Negus, PI; **Banks, Co-I**) 9/15/15-9/14/20
NIH/NIDA
Medications Development for Stimulant Abuse
The goal of these studies is to determine the role of opioid receptors in amphetamine-induced decreases in cocaine vs. food choice in rhesus monkeys.

UH2DA041146 (Janda; PI; **Banks Co-I**) 9/15/15-7/31/17
NIH/NIDA
Immunopharmacotherapy for Mitigating Opioid Addiction
The overall goal of this project is to develop and test novel immunotherapies for reducing the abuse-related behavioral effects of mu-opioid agonists heroin and prescription opioids in both nonhuman primate and rodent models.

R01 DA037287 Nicholson, **Banks (Co-PI)** 6/01/14-5/31/19
NIH/NIDA
Behavioral effects of NMDA antagonists/opioid agonist combinations
The overall goal of this project is to determine the relative selectivity of NMDA antagonists in combination with the mu-opioid agonist oxycodone to produce therapeutic effects in a preclinical model of thermal allodynia compared to other behavioral endpoints associated with abuse potential and opioid physical dependence in nonhuman primate models.

R01 DA033364 Lile (PI); **Banks (Co-I**) 02/01/13-12/31/16
NIH/NIDA
Medications Development for Cocaine: A Translational Approach in Monkey and Humans
Major Goals: The goal of these studies is to develop a novel translational behavioral model in nonhuman primates and humans for the evaluation of candidate medications. As a Co-I, I would participate in the nonhuman cocaine self-administration studies.

R01 DA031718 **Banks (PI)** (No Cost Extension) 9/15/12 – 7/31/17
NIH/NIDA
Treatment development for methamphetamine abuse

The overall goal of this project is to develop novel pharmacotherapies with both monoaminergic and nicotinic acetylcholingergic mechanisms for the treatment of methamphetamine abuse and dependence. This project involves both methamphetamine discrimination and methamphetamine self-administration in nonhuman primates.

Completed:

R21 DA036383 **Banks (PI)** 7/01/13-6/30/16

Effect of reinforcer type in cognitive behaviors

The overall goal of this project is to understand the effects of opioid dependence and withdrawal on cognitive behaviors maintained by either an opioid agonist or a food pellet.

Pending:

None

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Sidney Stevens Negus

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor; Dept. of Pharmacology and Toxicology; Virginia Commonwealth University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Virginia, Charlottesville, VA	BA	05/1981	English
University of North Carolina, Chapel Hill, NC	PhD	02/90	Neurobiology
Scripps Research Institute, San Diego, CA	Postdoc	08/91	Neuropharmacology
University of California, San Diego, CA	Postdoc	08/91	Anesthesiology

A. PERSONAL STATEMENT

I submit that I am qualified to serve as a co-sponsor on this F31 application for three reasons. **First**, I have participated for more than 20 years in research using laboratory animals to investigate candidate medications for treatment of drug abuse. This work has included studies conducted in nonhuman primates with the behavioral procedures proposed for this grant. **Second**, I also have more than 20 years of experience in research on the behavioral pharmacology of drugs that target opioid systems. In particular, I have experience in research in nonhuman primates with all the opioid agonists proposed for study in this application. **Third**, I have a history of training graduate students and postdoctoral fellows in general, and I already collaborate with both the applicant (Kathryn Schwienteck) and the sponsor (Dr. Matt Banks) of this F31 application in particular. I am also a co-investigator on the parent grant associated with this F31 application.

Citations below to recent review articles illustrate my experience with (a) medications development for drug abuse treatment in general and opioid abuse in particular, (b) my experience with preclinical research on behavioral pharmacology of opioids in nonhuman primates, and (c) my history of collaboration with the sponsor of this application.

1. Negus SS. Opioid antagonist effects in animal models related to opioid abuse: Drug discrimination and drug self-administration. In: Dean RL, Bilsky EJ, Negus SS, editors. Opiate Receptors and Antagonists: From Bench to Clinic. Humana Press, New York. 2009; p. 201-226.

2. Negus SS, Banks ML. Medications development for opioid abuse. In: Pierce RC, Kenny PJ, editors. Addiction. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 2013; pp. 199-210.: PMC3530034

3. Banks ML, Hutsell BA, Schwienteck KL, Negus SS. Use of preclinical drug vs. food choice procedures to evaluate candidate medications for cocaine addiction. Curr Treat Options Psychiatry, 2015; 2:136-150. PMC4441409

4. Negus SS, Henningfield J. Agonist medications for the treatment of cocaine use disorder. Invited "Circumspectives" Article: Neuropsychopharmacology, 2015; 40:1815-1825. PMC4839506

B. POSITIONS AND HONORS

Employment

9/81-8/85: United States Army; Noncommissioned Officer (final rank: E5)
8/85-2/90: Neurobiology Program, University of North Carolina, Chapel Hill, NC; Mentor-Linda Dykstra, PhD; Graduate Student
2/90-8/91: Dept. of Neuropharmacology, Scripps Research Institute, and Dept. of Anesthesiology, University of California, San Diego, CA; Mentors-George Koob, PhD, and Matthew Weinger, MD; Postdoctoral
8/91-2/93: Dept. of Pharmacology, University of Michigan, Ann Arbor, MI; Supervisor-James Woods, PhD; Research Investigator; Fulltime, Nontenured
2/93-9/07: Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, Belmont, MA; Supervisor: Nancy Mello, PhD; Assistant Professor (2/93-6/96); Associate Professor (6/96-9/07); Fulltime, Tenure Track
9/07-Present: Dept. of Pharmacology, Virginia Commonwealth University, Richmond, VA; Dept. Chair--William Dewey, PhD; Professor; Fulltime, Tenured

Honors

1993: Young Psychopharmacologist Award; American Psychological Association
1995: Milton Fund Award; Harvard Medical School
1995: Alfred Pope Young Investigator Award; McLean Hospital
1998: Joseph Cochin Young Investigator Award; College on Problems of Drug Dependence
2003: Board of Directors, College on Problems of Drug Dependence, (2003-07)
2003: Editorial Advisory Board, Psychopharmacology (2003-07)
2004: Editorial Advisory Board; Journal of Pharmacology and Experimental Therapeutics (2004-Present)
2006: Editorial Advisory Board: Journal of Experimental and Clinical Psychopharmacology (2006-Present)
2006: Editorial Advisory Board; Pharmacology, Biochemistry and Behavior (2006-Present)
2007: Lecturer, Department of Psychiatry, Harvard Medical School (2007-Present)
2012: Student Choice Award as Professor of the Year, Dept. Pharmacology, Virginia Commonwealth Univ
2015: Outstanding Departmental Teacher Award in Health Sciences Education, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University

Professional Societies and Public Advisory Committees

1988- Society for the Study of the Stimulus Properties of Drugs; Member
1988 Society for Neuroscience; Member
1992- American Association for the Advancement of Science; Member
1993- College on Problems of Drug Dependence; Member; Program Committee (1998-2001); Nominations Committee (2001-2002); Board of Directors (2003-2007); Committee on Abuse Liability Testing (2003-2007; Chair 2004-2007); Committee on Animals in Research (2007-present); CPDD representative to the Board of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC; 2011-)
1996- American Society for Pharmacology and Experimental Therapeutics; Member
1997-8 Member, Neuropharmacology Initial Review Group, National Institute on Drug Abuse
1998- American College of Neuropsychopharmacology; Member; Committee on Use of Animals in Research (2004-2007); Education and Training Committee (2016-18)
2000- American Pain Society; Member
2010-16 Standing or Ad Hoc Member: Somatosensory and Chemosensory Systems (SCS) Study Section, National Institutes of Health; Chair, 2013-14

C. Contributions to Science

I have published 184 peer-reviewed research manuscripts and 29 reviews and book chapters, and I have an H-index of 46 (Google Scholar, 7/25/16). Major themes of my research are summarized below, together with illustrative citations. *Indicates graduate student or postdoctoral trainees.

1. Preclinical Abuse Liability Assessment. Over the past 20 years, I have contributed to research to assess the abuse liability of opioids, stimulants, and drugs from other pharmacological classes. This work has included use of drug self-administration, drug discrimination, and intracranial self-stimulation procedures in both nonhuman primates and rodents. Citations for only review articles are provided here, but these reviews include citations to my primary research papers on this topic.

(a) Negus SS, Dykstra LA. Neural substrates of the reinforcing properties of opioid analgesics. In: Watson RR, editor. Focus on Biochemistry and Physiology of Substance Abuse, Vol. 1. Boca Raton: CRC Press; 1989. p. 211-42.
(b) Negus SS, Woods JH. Buprenorphine: reinforcing effects, discriminative stimulus effects and physical dependence liability. In: Cowan A, Lewis JW, editors. Buprenorphine. New York: Wiley-Liss; 1995. p. 71-101.
(c) Negus SS, *Banks ML. Making the right choice: Lessons from drug discrimination for research on drug reinforcement and drug self-administration. In: Glennon RA, Young R, editors. Drug Discrimination: Applications to Medicinal Chemistry and Drug Studies. John Wiley and Sons, Hoboken, NJ, 2011; pp. 361-388.
(d) Negus SS, *Miller LL. Intracranial self-stimulation (ICSS) to evaluate abuse potential of drugs. Pharmacol Rev, 2014; 66:869-917. PMC4081730

2. Medications Development to Treat Drug Abuse. Since 1993, I have made significant contributions to research on development of medications to treat stimulant and opioid abuse. Major themes in this work include development of procedures to evaluate candidate pharmacotherapies, strategies for analysis and interpretation of medication effects, and the importance of translational research.

(a) Mello NK, Negus SS. Preclinical evaluation of pharmacotherapies for treatment of cocaine and opiate abuse using drug self-administration procedures. Neuropsychopharmacology. 1996;14:375-424. PMID 8726752
(b) Grabowski J, Shearer J, Merrill J, Negus SS. Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. Addictive Behaviors (Special Issue: Crossing Boundaries: Implications of advances in basic sciences for the management of Addiction) 2004; 29:1439-1464. PMID 15345275
(c) Negus SS: Choice between heroin and food in non-dependent and heroin-dependent rhesus monkeys: Effects of naloxone, buprenorphine and methadone. J. Pharmacol. Exp. Ther., 2006; 317:711-723. PMID 16456085
(d) *Johnson AR, *Banks ML, Blough BE, Lile JA, Nicholson KL, Negus SS. Development of a translational model to screen medications for cocaine use disorder I: Choice between cocaine and food in rhesus monkeys. Drug Alcohol Depend, 2016; 165:103-110. PMC4939093

3. Neurobiology and Treatment of Pain. For the past decade, a portion of my effort has been devoted to research on expression, neurobiology and treatment of pain-related behavioral depression. This work has included development of novel behavioral procedures in rats and mice and validation of those procedures using opioids and other drugs.

(a) Negus SS, Vanderah TW, *Brandt MR, Bilsky EJ, Becerra L, Borsook D. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. Invited “Perspectives in Pharmacology” Article in J Pharmacol Exp Ther, 2006; 319:507-514. PMID 16751251
(b) Negus SS. Expression and treatment of pain-related behavioral depression. Lab Animal, 2013; 42:292-300. PMC Journal-In process. PMID 23877610
(c) Negus SS, *Altarifi AA. Mu, delta and kappa opioid agonist effects in novel assays of pain-depressed behavior. In: Ko H, Husbands, S, editors. Research and Development of Opioid-Related Ligands. American Chemical Society, Washington, DC. 2013, pp. 163-176. **DOI:** 10.1021/bk-2013-1131.ch009
(d) Negus SS, *Neddenriep B, *Altarifi AA, Carroll FI, *Leitl MD, *Miller LL. Effects of ketoprofen, morphine, and kappa opioids on pain-related depression of nesting in mice. Pain, 2015; 156:1153-1160. PMC4766843

4. Behavioral Pharmacology. Throughout my career, I have conducted studies to evaluate pharmacological mechanisms that mediate behavioral effects of opioids, stimulants, and drugs from other pharmacological classes. This work has been founded on quantitative approaches rooted in receptor theory and quantitative structure-activity relationships, and it includes analysis of potency, efficacy and time course of agonists,

quantitative antagonist studies with reversible and irreversible antagonists, and evaluation of drug interactions. Most recently, I have contributed to research investigating the pharmacology emerging cathinone analogs (i.e. “bath salts”).

(a) Negus SS, Burke TF, Medzihradsky F, Woods JH. Effects of opioid agonists selective for mu, kappa and delta opioid receptors on schedule-controlled responding in rhesus monkeys: antagonism by quadazocine. J Pharmacol Exp Ther 1993;267:896-903. PMID 8246165

(b) Negus SS, *Gatch MB, Mello NK, Zhang X, Rice K. Behavioral effects of the delta-selective opioid agonist SNC80 and related compounds in rhesus monkeys. J Pharmacol Exp Ther 1998; 286:362-75.

(c) *Banks ML, Rice KC, Negus SS. Antinociceptive interactions between mu-opioid agonists and the serotonin uptake inhibitor clomipramine in rhesus monkeys: role of mu agonist efficacy. J Pharmacol Exp Ther, 2010; 335:497-505. PMC2967402.

(d) *Bonano JS, *Banks ML, Kolanos R, *Sakloth F, Barnier M, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, Negus SS. Quantitative structure-activity relationship analysis of the pharmacology of para-substituted methcathinone analogues. Br J Pharmacol, 2015; 172:2433-2444. PMC4409897

5. Sex and Gonadal Hormones as Determinants of Drug Effects. An emerging NIH mandate promotes the use of both sexes in preclinical research with laboratory animals, and I have more than 15 years of experience in research to examine the pharmacology of stimulants and other drugs in gonadally intact male and female animals and in gonadectomized subjects treated with hormone replacements. Citations for a sample of this work are provided below.

(a) Negus SS, Mello NK. Opioid antinociception in ovariectomized monkeys: comparison to antinociception in males and effects of estradiol replacement. J Pharmacol Exp Ther 1999;290:1132-40. PMID 10454487

(b) *Caine SB, *Bowen CA, Yu G, *Zuzga D, Negus SS, Mello NK. The effect of gonadectomy and gonadal hormone replacement on cocaine self-administration in female and male rats. Neuropsychopharmacology, 2004; 29:929-942. PMID 14735136

(c) Mello NK, Negus SS, Knudson IM, Kelly M, Mendelson JH: Effects of estradiol on cocaine self-administration and cocaine discrimination by rhesus monkeys. Neuropsychopharmacology, 2008; 33:783-795. PMID 17507915

(d) Unpublished

link to publications: http://www.ncbi.nlm.nih.gov/pubmed/?term=Negus+ss

D. RESEARCH SUPPORT

Ongoing Research Support

R01DA026946 Negus, PI 7/01/09-6/30/20
NIH/NIDA
Medications Development for Stimulant Abuse
The goal of these studies is to evaluate pharmacologic and behavioral determinants of the utility of monoamine releasers as cocaine abuse treatment medications. One component of this project will be to evaluate effects of extended access to and withdrawal from cocaine self-administration on cocaine vs. food choice in rhesus monkeys.

R01NS070715 Negus, Corresponding MPI 9/30/09-7/31/19
NIH/NINDS
Neurobiology and Treatment of Pain
The goal of these studies is to evaluate the expression, pharmacologic modulation and underlying mechanisms of pain-related depression of ICSS in rats. These studies are founded on novel behavioral procedures developed by the PI.

R01DA030404 Negus, Corresponding MPI 7/1/11-4/30/16

NIH/NIDA
Endocannabinoid Modulation of Pain-Depressed Behavior
The goal of these studies is to evaluate the expression of pain-related behavioral depression in mice and to examine the role of endocannabinoids in modulation pain-depressed behavior.

R01DA033930 Negus, MPI 4/1/12-3/31/17
NIH/NIDA
Synthetic Cathinones: A New Class of Illicit Drugs Affecting DAT & SERT
The goal of these studies is to synthesize and evaluate derivatives of the psychostimulant cathinone, which are emerging as designer drugs of abuse. As co-PI, Negus would examine abuse-related neurochemical and behavioral effects of novel compounds.

R01DA033364 Lile, PI; Negus, co-I 2/1/13-1/31/17
Medications Development for Cocaine: A Translational Approach in Monkey and Human
The goal of these studies is to develop parallel cocaine choice procedures in humans and nonhumans primates that can be used to accelerate translational testing of candidate medications to treat drug abuse.

UH2DA041146 Janda, PI; Negus, co-I 9/15/15-7/31/17
Immunopharmacotherapy for Mitigating Opioid Addiction
The goal of these studies is to develop vaccines suitable for treatment of opioid addiction. Our portion of the grant focuses on evaluation of candidate vaccines in nonhuman primates.

Pending Research Support

Pending Support

Ongoing Trainee Grants

F30DA037649 Bonano, PI; Negus, Mentor 4/10/14-4/9/18
Mechanisms of Psychostimulant Abuse
Major Goals: To examine determinants of abuse-related behavioral and neurochemical effects of synthetic cathinones in rats.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Justin L. Poklis

eRA COMMONS USER NAME (credential, e.g., agency login) | eRA Commons User Name |

POSITION TITLE: Scientist II

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Virginia Commonwealth University	B.S.	08/1996	Chemistry

A. Personal Statement

I have over nineteen years of experience in analytical and forensic toxicology. Since graduating from Virginia Commonwealth University (VCU) I have used and maintained a variety of mass spectrometers. For the last nine years I have supervised, maintained and operated the Applied Bio systems 3200 Q trap with a turbo V source attached to a Shumadzu SCL system. This High Performance Liquid Chromatography tandem mass spectrometer (HPLC/MS/MS) located in the mass spectrometry laboratory in the Department of Pharmacology and Toxicology at VCU. I have conducted studies and published in a wide range of topics including methods for the determination of drugs, poisons and endogenous analytes in tissues and/or fluids, postmortem findings in drug deaths and drug metabolism. Currently I serve as the manager of spectrometry laboratory in the Department of Pharmacology and Toxicology at VCU. I have be directly involved in the development and validation of HPLC/MS/MS analytical methods and laboratory issues including specimen handling, preparation, analytic testing and laboratory safety. In my present position I have coordination the use of the HPLC/MS/MS, trained users and developed methods for the analysis of a wide variety of analytes using HPLC/MS/MS. The following four recent peer reviewed publications specifically highlight my experience in using HPLC/MS/MS. They also demonstrate the wide range of researchers that I have collaborated with and that have utilized the current HPLC/MS/MS in the mass spectrometry laboratory in the Department of Pharmacology and Toxicology at VCU.

1. **Poklis JL**, Clay DJ, Ignatowska-Jankowska BM, Zanato C, Ross RA, Greig IR, Abdullah RA, Mustafa MA, Lichtman AH, Poklis A. HPLC-MS-MS Determination of ZCZ-011, A Novel Pharmacological Tool for Investigation of the Cannabinoid Receptor in Mouse Brain Using Clean Screen FASt™ Column Extraction. **J Anal Toxicol**. 2015 May;39(5):353-358. PMC4542657

2. Banks ML, Hutsell BA, Blough BE, **Poklis JL**, Negus SS. Preclinical Assessment of Lisdexamfetamine as an Agonist Medication Candidate for Cocaine Addiction: Effects in Rhesus Monkeys Trained to Discriminate Cocaine or to Self-Administer Cocaine in a Cocaine Versus Food Choice Procedure. **Int J Neuropsychopharmacol**. 2015 Jan 24. PMC4458439

3. Wiley JL, Walentiny DM, Wright MJ Jr, Beardsley PM, Burston JJ, **Poklis JL**, Lichtman AH, Vann RE. Endocannabinoid contribution to Δ9-tetrahydrocannabinol discrimination in rodents. **Eur J Pharmacol**. 2014 Aug 15;737:97-105. PMC4110679

4. **Poklis JL**, Clay DJ, Poklis A. High-performance liquid chromatography with tandem mass spectrometry for the determination of nine hallucinogenic 25-NBOMe designer drugs in urine specimens. **J Anal Toxicol**. 2014 Apr;38(3):113-21. PMC3949485

B. Positions and Honors

Positions

June1996 - Dec 1996	Technologist, Scientific Testing Laboratory Richmond, Va
Jan 1997 - June 1997	[part-time]
Jan1997 - March 2001	Technologist, Toxicology Laboratories Medical College of Virginia Hospital, Richmond, VA
April 2001 - Jan 2003	Specialist Advance, Department of Pharmaceutics, Virginia Commonwealth University Richmond Va
June 2001 - Aug 2001	[part-time] Department of Pharmaceutics, Biopharmaceutical Laboratory Virginia Commonwealth University Richmond Va
Feb 2003 - Sept 2006	Specialist, Office of the Chief Medical Examiner, Chapel Hill, NC
Oct 2006 -Present	Specialist Advance, Department of Pharmacology & Toxicology, Virginia Commonwealth University Richmond Va

Honors

Diplomat of the American Board of Forensic Toxicology
Award Recipient of the Society of Forensic Toxicologist's Young Scientist Award, 2003
Society of Forensic Toxicology - Member
American Academy of Forensic Sciences - Member
International Association of Forensic Toxicologist –Member

C. Contribution to Science

My most significant contribution to science has been in the last nine years as the as the manager of spectrometry laboratory in the Department of Pharmacology and Toxicology. In this position I have been able to help support thought the use of the HPLC/MS/MS a wide range of principle investigators in their fund and unfunded research projects. The principle investigators of these projects would be able to speak to the overall contribution to science. I can only state the contribution to science for the two projects that I have been most involved in. Both projects evaluated new designer drugs and included development of new methods for the detection and quantification use HPLC/MS/MS.

The first of these projects involved **the class of designer drugs known as cannabimimetics**. These drugs included compound such as JWH-018, JWH-073 and CP-47,497. They produce effects similar to Tetrahydrocannabinol (THC) and work on the CB1 and CB2 receptors. They were unregulated and sold as herbal incense products often labeled as "not for human conceptions". There was little to no published data on the effects, absorption, metabolism, and elimination when they became publicly available. The HPLC/MS/MS methods that I developed in collaboration with principle investigators resulted in providing analytical methods for their detection, biological biomarkers to determine use and pharmacological data on their effects (a,b,c,d).

a. **Poklis JL**, Amira D, Wise LE, Wiebelhaus JM, Haggerty BJ, Poklis A. Detection and disposition of JWH-018 and JWH-073 in mice after exposure to "Magic Gold" smoke. **Forensic Sci Int**. 2012 Jul 10;220(1-3): PMC3677765

b. **Poklis JL**, Amira D, Wise LE, Wiebelhaus JM, Haggerty BJ, Lichtman AH, Poklis A. Determination of naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) in mouse blood and tissue after inhalation exposure to 'buzz' smoke by HPLC/MS/MS. **Biomed Chromatogr**. 2012 Nov;26(11):1393-8. PMC3697740

c. Wiebelhaus JM, **Poklis JL**, Poklis A, Vann RE, Lichtman AH, Wise LE. Inhalation exposure to smoke from synthetic "marijuana" produces potent cannabimimetic effects in mice. **Drug Alcohol Depend**. 2012 Dec 1;126(3):316-23. PMC3501554

d. Samano KL, **Poklis JL**, Lichtman AH, Poklis A. Development of a high-performance liquid chromatography-tandem mass spectrometry method for the identification and quantification of CP-47,497, CP-47,497-C8 and JWH-250 in mouse brain. **J Anal Toxicol**. 2014 Jul-Aug;38(6):307-14. PMC4148611

The second collaborative project resulted in the developed **HPLC/MS/MS methods for a new series of designer hallucinogens**, the "NBOMe" (dimethoxyphenyl-N-[(2-methoxyphenyl)methyl]ethanamine) derivatives. These drugs were unregulated and sold to the public. They are now known to cause adverse reactions including seizure, violent, bizarre and/or self-destructive behaviors. These reactions were first published in peer reviewed papers that I co-author (a,b). These papers included the first published methods for their detection and quantification in blood, fluid and tissue specimens as part of case reports involving overdoses of the two most popular NBOMe derivatives, 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl]ethanamine (a,c,d) and 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl]ethanamine (b).

a. Rose SR, **Poklis JL**, Poklis A. A case of 25I-NBOMe (25-I) intoxication: a new potent 5-HT2A agonist designer drug. **Clin Toxicol (Phila)**. 2013 Mar;51(3):174-7. PMC4002208

b. **Poklis JL**, Nanco CR, Troendle MM, Wolf CE, Poklis A. Determination of 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine (25B-NBOMe) in serum and urine by high performance liquid chromatography with tandem mass spectrometry in a case of severe intoxication. **Drug Test Anal**. 2014 Jul-Aug;6(7-8):764-9. PMC4002638

c. **Poklis JL**, Charles J, Wolf CE, Poklis A. High-performance liquid chromatography tandem mass spectrometry method for the determination of 2CC-NBOMe and 25I-NBOMe in human serum. **Biomed Chromatogr**. 2013 Dec;27(12):1794-800. PMC4077323

d. **Poklis JL**, Devers KG, Arbefeville EF, Pearson JM, Houston E, Poklis A. Postmortem detection of 25I-NBOMe [2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine] in fluids and tissues determined by high performance liquid chromatography with tandem mass spectrometry from a traumatic death. **Forensic Sci Int**. 2014 Jan;234:e14-20. PMC3969735

Both of these projects have help make the scientific and public communities aware of the dangers of these designer drugs.

URL to most of my peer-reviewed publications
http://www.ncbi.nlm.nih.gov/pubmed/?term=poklis+j

D. Research Support

Ongoing Research Support

P30DA033934 Alphonse Poklis (P.I) 10/1/13-12/13/17
National Institute of Health "Analytical Core"
The Bioanalytical Core Laboratory provides state of the art and innovative bioanalytical expertise and techniques for the quantification of drugs and their metabolites, endogenous chemicals and other molecules of biological significance in new collaborative studies with the other cores in the center and all the funded drug abuse biomedical researchers at this and neighboring universities.
Role: Co-Investigator

NIJ-2014-R2-CX-K010 Michelle R. Peace (P.I) 1/12/14-12/13/16

National Institute of Justice "Characterization and Abuse of Electronic Cigarettes"
The Efficacy of a Personal Vaporizer as an Illicit Drug Delivery System
Role: Co-Investigator

R21DA036809 Ziva D. Cooper (P.I) 7/1/15-7/31/16
Research Foundation for Mental Hygiene, Inc
"Synthetic of smokable synthetic Cannabinoids (Spice) in Humans"
Study provides techniques, methods and models for future research with this class of compounds and can be extended to other chemical classes of cannabinoids that may emerge.
Role: Co-Investigator

PHS Fellowship Supplemental Form

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction

1. Introduction
(RESUBMISSION)

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training*	160802_BackgroundsGoals_KLS.pdf

Research Training Plan Section

3. Specific Aims*	160802_SPECIFICAIMS_KLS.pdf
4. Research Strategy*	160802_ResearchPlan_KLS.pdf
5. Respective Contributions*	Respective_Contributions_160731_KLS.pdf
6. Selection of Sponsor and Institution*	160801_Sponsorselection_KLS.pdf
7. Progress Report Publication List (RENEWAL)	
8. Training in the Responsible Conduct of Research*	Responsible_Conduct_of_Research_160731_KLS.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	160731_MentorStatement_KLS.pdf
10. Letters of Support from Collaborators, Contributors and Consultants	160727_Letters_of_support.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	160729_Institutional_Environment_KLS.pdf

Other Research Training Plan Section

Human Subjects

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? ☐ Yes ☑ No

12. Human Subjects Involvement Indefinite?

13. Clinical Trial?

14. Agency-Defined Phase III Clinical Trial?

15. Protection of Human Subjects

16. Data Safety Monitoring Plan

17. Inclusion of Women and Minorities

18. Inclusion of Children

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☑ Yes ☐ No

19. Vertebrate Animals Use Indefinite? ☑ Yes ☐ No

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20. Are vertebrate animals euthanized? If "Yes" to euthanasia Is method consistent with American Veterinary Medical Association (AVMA) guidelines? If "No" to AVMA guidelines, describe method and provide scientific justification	☐ Yes ☑ No
21. Vertebrate Animals	160728_Vertebrate_animals_KLS.pdf

Other Research Training Plan Information

22. Select Agent Research

23. Resource Sharing Plan

24. Authentication of Key Biological and/or Chemical Resources

PHS Fellowship Supplemental Form

Additional Information Section

25. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ☐ Yes ☑ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

26. Alternate Phone Number: 804-828-8466

27. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):
PHD: Doctor of Philosophy		05/2019

28. Field of Training for Current Proposal*: 180 Pharmacology, Human & Animal

29. Current Or Prior Kirschstein-NRSA Support?* ☐ Yes ☑ No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)

30. Applications for Concurrent Support?* ☐ Yes ☑ No

If yes, please describe in an attached file:

31. Citizenship*

U.S. Citizen — U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen
- ☐ With a Permanent U.S. Resident Visa
- ☐ With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, please also check here. ☐

32. ☐ Change of Sponsoring Institution — Name of Former Institution:*

PHS Fellowship Supplemental Form

Budget Section

All Fellowship Applicants:

1. Tuition and Fees*:

☐ None Requested ☑ Funds Requested

Year 1	$16,000.00
Year 2	$16,000.00
Year 3	$2,667.00
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	$34,667.00

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary: Amount | Academic Period | Number of Months

3. Stipends/Salary During First Year of Proposed Fellowship:

a. Federal Stipend Requested: Amount | Number of Months

b. Supplementation from other sources: Amount | Number of Months

Type (sabbatical leave, salary, etc.)

Source

Appendix

APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

A. Doctoral Dissertation and Research Experience:

My first exposure to research was during my undergraduate education at the University of Pittsburgh. Part of the neuroscience degree requirement included one semester of an independent research component and students were instructed to select a faculty member to work with whose research was deemed interesting to the student. After examining the neuroscience faculty bio's, I immediately found interest in the work of Charles Bradberry, PhD. His work focused on studying the neurobiology of drug addiction and cognitive deficits in nonhuman primates. I joined Dr. Bradberry's lab in 2009 and spent three semesters working as an undergraduate research assistant in the nonhuman primate laboratory. During my time in his laboratory, I visited the lab twice a week before class and ran the self-administration experiments that were part of a graduate student's project studying potential cognitive deficits seen after chronic cocaine use. The procedure required using a pole and collar system to remove the monkeys from their cages and guide to them to their restraint chairs before transporting them down the hallway to operant chambers in which they completed their self-administration behavioral sessions. I assisted in conducting the experiments, data collection, and in the maintenance of intravenous port-a-catheters. The entire procedure was extremely labor intensive, and at first quite intimidating, especially since this was my first exposure to nonhuman primates. However, over time, the monkeys grew to recognize and trust me and I began to understand their individual behaviors which helped me to gain confidence in working so closely with them. I also attended weekly laboratory meetings which helped me to understand the science behind the project that I was assisting with. The experience in the laboratory was extremely interesting to me and has been quite a defining point in my training. Another pivotal experience at the University of Pittsburgh were two elective classes that I took: Drugs and Behavior and Psychiatric Disorders and Brain Function. Although I had always been interested in the fascinating world of drugs of abuse, these two classes provided me with formal instruction about the pharmacology of drugs of abuse and in particular, substance-use disorders. I believe that the combination of these classes and the experience in Dr. Bradberry's laboratory provided a solid foundation for me to transition into the next series of events that have ultimately led me to my current position as a graduate student studying drugs of abuse in the VCU Department of Pharmacology and Toxicology.

As I prepared to graduate from the University of Pittsburgh, I felt a deep desire to learn more about pharmacology and at the time, pursuing a career in pharmacy seemed to be the solution. Therefore, I continued my education by attending Virginia Commonwealth University School of Pharmacy in Richmond, Virginia. The coursework in pharmacy school introduced me to hundreds of drugs and taught me about the clinical implications of the compounds. However, I found that the pharmacology sections of each clinical module was extremely fascinating and the material seemed intuitive to me. During the first two years of pharmacy school, I obtained many clinical skills but also realized that I actually could not see myself working as a pharmacist long-term. Therefore, I found myself seeking different avenues, and spoke to Imad Damaj, PhD from the VCU Department of Pharmacology and Toxicology after class one day who insisted that I visit the department at some time. At this point, I investigated the department and discovered that medications development testing for substance-use disorders in nonhuman primates was prominent at the university. This insight immediately attracted me and I contacted the PI, Dr. Matthew Banks, of one of the nonhuman primate laboratories so that I could learn more about their research. Next, I applied for a fellowship from the School of Pharmacy that I received, and which provided me with funds for the summer to work with Dr. Matthew Banks in the self-administration monkey choice laboratory. During this time, Dr. Banks taught me about designing experiments, the importance of being up-to-date on literature, and about the peer review process and manuscript publication. We worked together on a project that examined the subchronic administration of nicotine and termination from nicotine treatment on cocaine self-administration in nonhuman primates. Dr. Banks also allowed me to observe an aseptic catheter insertion surgical procedure and I truly felt like a member of the laboratory as I began to attend the biweekly lab meetings. As part of the summer fellowship, I also gained experience in presenting my project at a pharmacy school research seminar, and subsequently prepared a poster and presented the poster at the School of Pharmacy Research and Career day. My poster presentation won best PharmD student poster award and I began working on a manuscript with Dr. Banks that was eventually submitted for publication and accepted. My experience working in the laboratory during the summer fellowship lead me to decide to pursue my PhD after I completed pharmacy school.

Since early 2015, I have been an active member of the Banks laboratory. I worked on a second project with Dr. Banks in the self-administration laboratory that examined the effects of monoamine reuptake inhibitors and releasers on self-administration of methamphetamine in nonhuman primates. I had more of an independent role on this project and made decisions as to when animals were ready to receive treatment regiments based on their baseline behavior. After the experiment was completed, I wrote a manuscript with Dr. Banks and submitted it for publication. I also presented the data at an external annual laboratory exchange with other nonhuman primate laboratories that is held at either Wake Forest University or Emory University annually. It was my first time attending this exchange and I had a very positive experience. I was able to tour the Yerkes nonhuman primate research center, and also met and formed connections with other graduate students who conduct nonhuman primate research.

Upon starting graduate school, I rotated with Dr. Steve Negus in his intracranial self-stimulation (ICSS) rodent laboratory. I learned to surgically and ascetically implant a bipolar electrode in the medial forebrain bundle at the level of the lateral hypothalamus in a rat's brain. Furthermore, I learned how to train rats on a frequency-rate behavioral program and how to conduct acute, chronic, and cumulative dosing experiments and time course determinations to examine drug effects on ICSS. I worked on two projects while I was on rotation with Dr. Negus. First, I examined the abuse-related effects of synthetic cathinone-analogs on intracranial self-stimulation. Data from this project will be part of a manuscript with data from collaborator laboratories that have examined the same compounds in different assays. Second, I examined the acute and chronic effects of $GABA_A$ positive allosteric modulators (PAMs) on ICSS. This project has provided me with the opportunity to present the data at several national and local conferences. I am currently in the process of preparing a manuscript that will be submitted for publication.

In conclusion, my research experiences in Dr. Bradberry's laboratory piqued my interest in drug abuse research and trained me to conduct difficult nonhuman primate research, while my pharmacy school career provided me with an understanding of pharmacology and clinical experiences. The time I spent in Dr. Banks laboratory has instructed me how to study potential medication effects on the reinforcing properties of abused drugs, while my time in Dr. Negus laboratory has taught me how to study drugs of abuse in preclinical rodent models of abuse liability. As I carry out this proposal and work towards my second doctoral degree, the collective resources from the Banks (Sponsor) and Negus (Co-Sponsor) laboratories will refine my research skills and help facilitate my transition to conduct drug abuse research as an independent scientist.

B. Training Goals and Objectives:

My long-term career goal is to become an independent drug abuse researcher at an NIH/NIDA funded institution and receiving a NRSA award will be a pivotal step in achieving this goal. While I have been exposed to studies that have examined the abuse liability of novel compounds, and potential treatments for substance use-disorders, this proposal will introduce me to new techniques and enable me to thoroughly examine a potential treatment for heroin addiction. Under the direction of Dr. Matthew Banks (Sponsor) and Dr. Steve Negus (Co-Sponsor) I will gain valuable experience studying new behavioral techniques in nonhuman primates that may be used to assess abuse-related subjective effects of heroin and antinociceptive properties of heroin. Furthermore, I hope that this proposal will increase my independence and confidence as a drug abuse researcher.

In terms of my training, the goal of this fellowship is to enhance and refine my knowledge of drug abuse pharmacology. It is intended that these experiments and training opportunities will supplement my current ability to conduct behavioral analyses with new techniques that I will be able to use throughout my career. Since my research interests include the development and evaluation of novel medications for the treatment of substance-use disorders, this NRSA award will provide me with the opportunity to conduct these types of experiments first-hand. The proposal will also introduce me to new fields of research including the potential utility of immunopharmacotherapy for drug addiction, and also the field of pain pharmacology. As part of my training, I am enrolled to take a course taught by Steve Negus, PhD next semester titled PHTX 691: Pain Pharmacology. Furthermore, my sponsor is planning on sending me to Scripps Research Institute to meet with the chemists who have synthesized the vaccine we are testing. It is expected that I will learn how the vaccine is synthesized and tested in their laboratory. Furthermore, I intend to learn how antibody levels are quantified since we will be sending blood samples to them for analysis. The NRSA award is key in developing my ability to write and submit grants which will be of extreme importance in my next career step, as I will be able to take what I learned from

this grant application and apply it to future applications in my postdoctoral and independent research careers. Certain techniques that this proposal will teach me include pharmacokinetic analysis using mass spectrometry, programming notation to write behavioral programs, and new behavioral procedures including drug discrimination and a warmwater tail-withdrawal procedure. This proposal will also provide me with an opportunity to train monkeys on operant behavioral procedures and I will learn how to monitor and analyze monkey's progress throughout the training and testing phase of discrimination. Furthermore, I will learn how to train monkeys to sit in restraint chairs and collect blood for antibody titers and pharmacokinetic analysis. I will spend time in the VCU Mass Spectroscopy laboratory with Justin Poklis who will train me in techniques used to analyze blood samples for heroin and metabolites.

The scientific goals of this fellowship include evaluating whether a novel heroin vaccine is effective in blunting the abuse-related subjective effects of heroin and whether it is able to block antinociceptive properties of heroin, while still allowing for antinociceptive properties of other compounds. These proposed studies are the first steps towards the ultimate goal of determining whether the heroin-TT conjugate vaccine would be an efficacious treatment for heroin addiction in a clinical population.

With the financial support of a NRSA award, I will be able to share my findings with the drug abuse research community at scientific meetings and through future publications. Additionally, the award will enable me to become a member of the College on Problems of Drug Dependence (CPPD) and also a member of American Society for Pharmacology and Experimental Therapeutics (ASPET). With funding from the NRSA award, I will be able to attend these meetings and also present my findings to fellow drug abuse researchers. I hope to form connections with other drug abuse researchers so that I can share ideas and formulate new ones. My future plans include the possibility of transitioning into human drug abuse research, and I have already formed connections with researchers in this field including Redacted by agreement I hope that I will continue to see these two individuals at future conferences so that I might learn more about conducting human drug abuse research.

In conclusion, I believe that training in the techniques and fields of research discussed above will help meet my career goal of becoming an independent drug abuse researcher. The research environment that I will be conducting these studies in will provide me with the perfect resources and mentorship to transition me to my future career as a drug abuse scientist.

C. Activities Planned Under this Award:

Year 01: The earliest that funding will be available will be April 2017 after submitting in the August 2016 cycle. All primary coursework will be complete by December 2016, so the majority of my time will be spent conducting the proposed research starting in January 2017. During the semester, I will attend up to three hours of weekly seminars that are sponsored by the department. I plan on attending and presenting data at the annual conferences Behavior, Biology and Chemistry (BBC): Translational Research in Addiction in the early spring, Virginia Academy of Science (VAS) conference held in late spring, and the College on Problems of Drug Dependence (CPDD) held in the early summer. Furthermore, our lab and other nonhuman primate labs from Wake Forrest, Emory University, University of Mississippi congregate once a year to present data at the annual lab exchange which I will also attend and present data at. A benchmark for this year will be passing my oral competencies and advancing on to PhD candidacy.

Year 02: The majority of time will be spent conducting research. Career development workshops are held often by the VCU Career Services, some of which I have already taken advantage of, including a visit to tour the NIH. However, I plan on spending more time investigating resources they provide in order to find a postdoc position later on in my training. I plan on applying to attend the 2017 Lindau Noble Laureate Conference held annually in Germany in 2017. Furthermore, I will continue to meet with my advisor weekly and attend biweekly lab meetings and also present data at the above mentioned conferences.

Year 03: During my final year of the proposed research project, I plan on spending most of my time finishing up experiments. I will continue to present data from the findings at the above mentioned conferences and also at biweekly lab meetings. Major benchmarks for this year include writing and defending my PhD dissertation, publishing 2 first authored manuscripts and also securing a postdoc position.

The chart presented below provides the total effort for each year of funding:

	Pre-Funding period	Year-01	Year-02	Year-03
Coursework				
Advanced Pain Elective	EFFORT			
Student & Faculty Seminars				
Mentored Research Activity				
Conducting research	EFFORT			
Learning new techniques				
Lab meetings & Sponsor Meetings				
Career Development activities				
Present at meetings & external joint lab exchanges	EFFORT			
Write & Submit grant application				
Attend career development workshops				
Apply & Attend 2017 Lindau Nobel Laureate Meeting				
Benchmarks				
2 first authored manuscripts				X
Pass oral competencies		X		
Write and defend PhD Dissertation				X
Secure Postdoc position				X

SPECIFIC AIMS

Heroin addiction is a significant public health issue, and the recent increases in rates of heroin abuse and overdose have further compounded this public health issue. Although there are FDA-approved mu-opioid agonist-like medications (e.g. methadone and buprenorphine) for heroin addiction, both medications also possess the undesirable side-effect of abuse liability. FDA-approved antagonist pharmacotherapies, such as naltrexone, are also available, yet patient compliance is poor. Furthermore, antagonist-based therapies possess the undesirable risk of antagonizing both the abused mu-opioid agonist and mu-opioids utilized for analgesia. Overall, these examples highlight the need for effective pharmacotherapies with reduced undesirable effects. One novel approach for treating heroin addiction may be a selective vaccine-based therapy or immunopharmacotherapy. Accordingly, this application proposes preclinical studies in male and female monkeys to determine the efficacy and specificity of a heroin-TT conjugate vaccine on the abuse-related subjective-like and antinociceptive effects of heroin, its metabolites 6-acetylmorphine (6-AM) and morphine, and other structurally distinct mu-opioid agonists.

Vaccine-based pharmacotherapies have been developed as selective therapeutics to attenuate the abuse-related behavioral effects without directly engaging the neurocircuitry involved in mediating these abuse-related effects. Preclinical drug discrimination procedures provide an efficient method to evaluate vaccine efficacy and specificity and provide a dependent measure that is relatively insensitive to vaccine or drug-induced rate-altering effects. Another proposed advantage of immunopharmacotherapy approaches is their specificity for the target abused drug. Specificity may be important for heroin abusers who might also need medical treatment for acute pain. For example, methadone-maintained heroin abusers often experience hyperalgesia following mu-opioid administration for pain. To test this hypothesis, this application will determine the antinociceptive effects of heroin and its metabolites and structurally dissimilar mu-opioid agonists in a warmwater tail-withdrawal procedure before and after vaccination with the candidate heroin vaccine. Overall, we propose the evaluation of a heroin-based immunopharmacotherapeutic would be significant given the rising prevalence of opioid addiction and the undesirable effects associated with current FDA-approved pharmacotherapies.

Preclinical immunopharmacotherapy evaluation in nonhuman primate models represents a critical component in the vertical translation drug development process. Nonhuman primates are proposed as research subjects for the following two reasons. First, there are species differences in opioid system biology, and in particular, both opioid pharmacokinetics and abuse-related behavioral effects that may influence immunopharmacotherapy potency or efficacy. To date, the evaluation of a candidate heroin vaccine has not been examined in nonhuman primates. Second, there may be species differences in the potency or efficacy of immunopharmacotherapies to elicit antibody production and/or undesirable immune response. Thus, from a toxicological and safety experimental perspective, nonhuman primates provide a clinically translatable bridge from rodent to human studies. Accordingly, this proposal is organized into three specific aims:

Aim 1. Determine heroin-TT conjugate vaccine efficacy and selectivity on mu-opioid agonist antinociceptive effects in male and female monkeys. Behavioral studies will be conducted to determine the efficacy and selectivity of a heroin vaccine on the fentanyl-like discriminative stimulus effects of heroin and its primary metabolites 6-acetylmorphine (6-AM) and morphine in a two-key food-reinforced fentanyl vs. saline drug discrimination procedure. We hypothesize that heroin vaccine administration will attenuate the potency of heroin and 6-AM, but not morphine and the structurally distinct mu-opioid agonist fentanyl to produce fentanyl-like discriminative stimulus effects.

Aim 2. Determine heroin-TT conjugate vaccine efficacy and selectivity on mu-opioid agonist antinociceptive effects in male and female monkeys. Behavioral studies will be conducted to determine the efficacy and selectivity of a heroin vaccine on the antinociceptive effects of heroin and its primary metabolites 6-AM and morphine in a warm water tail withdrawal procedure. We hypothesize that heroin vaccine administration will attenuate the potency of heroin and 6-AM, but not morphine and the structurally distinct mu-opioid agonist fentanyl to produce antinociceptive effects.

Aim 3. Correlate heroin pharmacokinetics with behavioral effects. Parallel heroin pharmacokinetic studies will be conducted before and after vaccine administration to correlate heroin metabolite levels with behavioral dependent measures in both the drug discrimination and warm water tail withdrawal procedures. We hypothesize that 6-AM plasma levels will correlate with heroin abuse-related and antinociceptive effects before vaccine administration, but not after vaccine administration. This expected pharmacokinetic profile would be consistent with the vaccine sequestering heroin in the circulatory system for elimination.

RESEARCH STRATEGY

Significance

Heroin abuse and addiction is a significant public health problem and national survey data estimate approximately 914,000 people aged 12 or older used heroin in 2014 [9,10,12-14]. In addition, the number of deaths related to heroin overdose have more than quintupled from 2000 to 2014 [10,11]. Healthcare costs associated with these hospitalizations from heroin overdose and infections have tripled from 2002 to 2012 [8]. Heroin use increases the spread of hepatitis B/C and HIV [5-7], and intravenous users are at risk for developing endocarditis and other bacterial infections [3,4]. Although Food and Drug Administration (FDA)-approved agonist (e.g. methadone) and antagonist-based (e.g. naltrexone) medications exist for heroin addiction treatment, the staggering increase in heroin use in the past decade highlights a need to develop new options for the treatment of heroin addiction [1,2,15]. Moreover, agonist-based therapies possess abuse liability, diversion potential, and can result in iatrogenic dependence or toxicity from high doses [15, 22-30]. With antagonist-based therapies, patient compliance and treatment retention are quite difficult to maintain [31-38]. Antagonist therapies also possess the risk of antagonizing the effects of all mu opioid agonists, some of which may be necessary to treat acute pain. An emerging treatment strategy that is proposed in this fellowship application is a selective vaccine-based therapy or immunopharmacotherapy [39-43]. The proposed heroin vaccine works by triggering an immune response that results in production of antibodies specific for the heroin molecule. After heroin administration, the heroin-specific antibodies will bind to the drug molecule and prevent the translocation into the brain. Immunopharmacotherapy advantages over available current FDA-approved treatments could be that a heroin-vaccine would not produce abuse-related effects alone nor nonselectively block all mu-agonist effects [72]. In this proposal, we will determine if the heroin-TT conjugate vaccine will alter abuse-related subjective effects of heroin in a two-key food-reinforced drug discrimination procedure in male and female nonhuman primates. Second, we will determine if the vaccine will blunt antinociceptive properties of heroin or other opioids in a warmwater tail-withdrawal procedure in the same nonhuman primates to compare vaccine effects across different behavioral dependent measures that have distinct biological mechanisms. Lastly, we will correlate pharmacokinetic parameters to the behavioral outcomes seen in both procedures to improve understanding of pharmacokinetic-pharmacodynamic relationships between drug discrimination and thermal antinociception produced by mu opioid agonists. Utilizing nonhuman primates in this proposal has a clear advantage as nonhuman primates and humans have a similar opioid subjective-effect and pharmacokinetic profile that may influence immunopharmacotherapy efficacy or potency.

Scientific Premise: Immunopharmacotherapies are being investigated as potential treatments for substance-use disorders. While candidate vaccines for cocaine substance-use disorders have advanced to clinical trials, one potential reason for poor clinical efficacy could be due to a lack of preclinical evaluation in the appropriate animal models [85,86]. Although immunotherapies have been extensively evaluated in rodent models, there are a paucity of studies determining vaccine treatment effects in nonhuman primate models of drug abuse. For example, there is only one study from 1970's that has studied a vaccine to alter effects of heroin self-administration in monkeys [84]. To address this gap in our scientific knowledge, we propose to study a novel heroin-TT conjugate vaccine in two behavioral procedures that have been extensively used in nonhuman primates to study subjective-like and antinociceptive effects of mu-opioid agonists. Preliminary data from our laboratory in a behavioral assay of schedule-controlled responding show the proposed heroin-TT conjugate vaccine shifted the potency of heroin to decrease operant rates of responding by approximately 4-fold in male rhesus monkeys and demonstrate feasibility of the proposed studies.

Approach

Rigorous Experimental Design: All dose effect functions in aims 1 and 2 will be double-determined alone and in combination with naltrexone (positive control). Both aims also include testing with a known negative control to assess the specificity of the behavioral assays. Furthermore, all experiments include testing with a control vaccine prior to testing with the heroin-TT conjugate vaccine. Considering the complete within-subject design, a sample size of 6 monkeys (3 females and 3 males) will provide 80% statistical power to detect a minimum difference in behavioral performance with an expected effect size of 1.15. These calculations are based on a 5% alpha level. Drugs in each aim will be tested in a randomized order. Although blinding will not be utilized, the dependent measure is based on animal's performance and thus is minimally susceptible to subjective biases. In the event of missing data, our lab has experience in dealing with missing data points due of rate-suppressant drug effects [56].

Consideration of Relevant Biological Variables: Relevant biological variables have been considered in the experimental design. Our research plan includes a balance of male and female rhesus monkeys to address the recent NIH mandate and female menstrual cycles will be monitored throughout the studies. Female menstrual cycles will be monitored daily throughout the study. All monkeys are young adults and have been determined to be in good health by institutional veterinarians.

Preliminary findings

Training of fentanyl (0.01 mg/kg, IM) as a discriminative stimulus in a representative male rhesus monkey (n=1) (Figure 1). Data show training results from the last 7 training sessions during which either saline or fentanyl was designated as the training discriminative stimulus. Top panel shows percent injection appropriate responding on the Y-axis and session number on the X-axis. The horizontal lines denote *a priori* acquisition criteria for both first reinforcer behavioral allocation (%1st Reinf) and overall session behavioral allocation (%AR). Bottom panel shows rates of operant responding in responses per second on the Y-axis and session number on the X-axis. These data show stable rates of responding during both fentanyl and saline training sessions. These preliminary data demonstrate feasibility to train fentanyl as a discriminative stimulus in our laboratory.

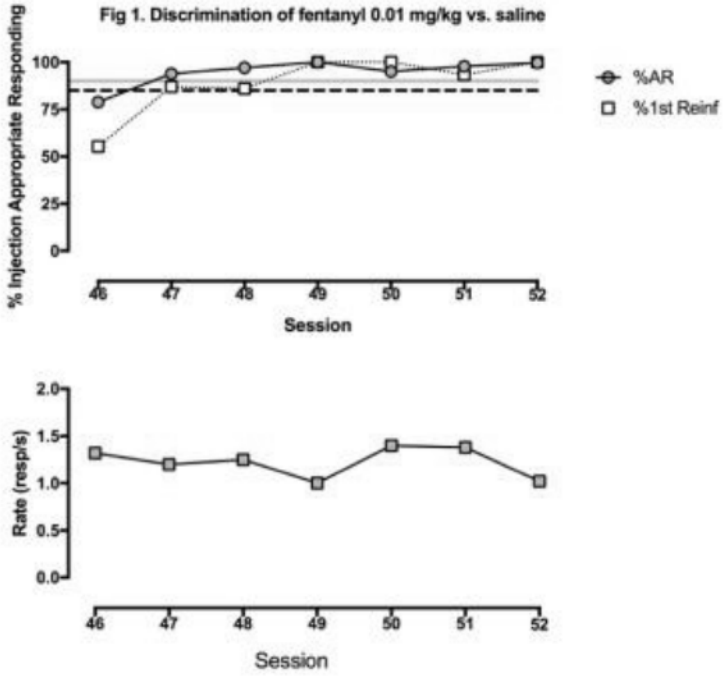

Fig 2. Effects of heroin vaccine on rates of responding

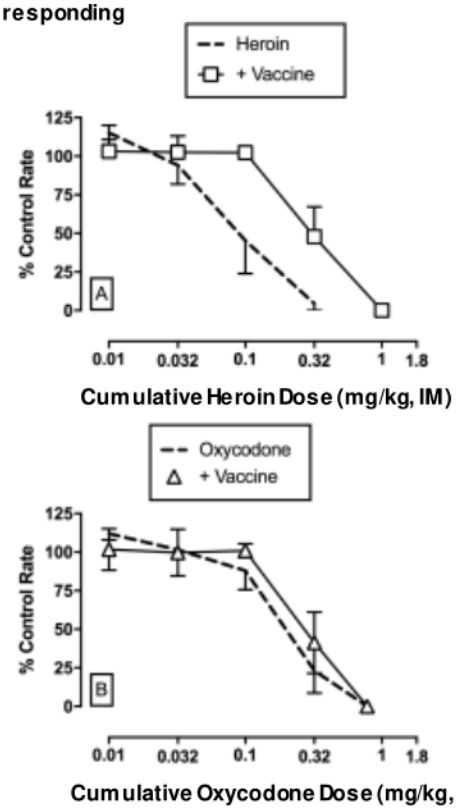

Figure 2 (Left) shows the effects of a heroin-TT conjugate vaccine on the rate-suppressant effects of heroin (Panel A) and oxycodone (Panel B) in an assay of schedule controlled responding in male rhesus monkeys (n=4). Y-axis is percent control rate of responding and x-axis is the cumulative drug dose (mg/kg, IM) administered. Before vaccine administration, both heroin and oxycodone produced a dose-dependent decrease in rates of operant responding. After vaccine administration, the heroin potency to decrease rates of operant responding was shifted approximately 4-fold whereas the oxycodone potency was unaltered. These preliminary results demonstrate feasibility of the proposed heroin-TT conjugate vaccine to alter the behavioral effects of heroin in rhesus monkeys and support the studies proposed in this application determining the effects of a heroin-TT conjugate vaccine on the abuse-related subjective effects of heroin in male and female rhesus monkeys.

Specific Aim 1: Determine heroin-TT conjugate vaccine efficacy and selectivity on mu-opioid agonist abuse-related subjective-like effects in male and female monkeys.

Rationale. Drug discrimination is a well-established behavioral procedure to interrogate the abuse-related subjective effects of drugs, and we propose to use the procedure for three reasons. First, drug discrimination allows for a sensitive measure of interoceptive, centrally-mediated drug effects [17,20,44,45,48]. For example, a subject could be trained to respond on one key after administration of the training drug and respond on a different key after administration of vehicle. Once trained, generalization tests may be conducted, in which a test compound is given, and the animal's overall behavioral allocation on a certain key allows for conclusions to be drawn regarding the potency and efficacy of a drug to produce abuse-related subjective-like effects. It is well established that opioid compounds will substitute for opioid training drugs, and thus, drug discrimination will provide us with a reliable

measure to study vaccines effects on the generalization of heroin and its metabolites while allowing for the training drug to function as a structurally dissimilar mu-opioid agonist control [18,19]. Second, drug discrimination provides a dependent measure of behavioral allocation and thus drug discrimination provides a means to measure abuse-related subjective effects of drugs that is less sensitive to any vaccine or drug-induced rate-altering effects [49]. Third, drug discrimination studies conducted in nonhumans reliably mirror subjective effects of abused-drugs in humans and therefore, results obtained in our studies may provide important data regarding vaccine efficacy to alter interoceptive effects in humans, the ultimate population of interest [46,47]. Therefore, a drug discrimination procedure will provide us with a sensitive preclinical measure, relatively independent of response rate that may have clinical implications. In Aim 1, we will determine the heroin-TT conjugate vaccine's effectiveness in altering the discriminative stimulus effects of heroin in monkeys trained to discriminate fentanyl from saline. Although there are a number of training drugs that we could have chosen, we chose fentanyl for two reasons. First, fentanyl has been demonstrated by two other studies to be trainable as a discriminative stimulus in monkeys [52,53]. Second, the heroin-vaccine we are testing recognizes the 6-acetyl group that is present on the heroin molecule. Fentanyl does not possess the 6-acetyl group, and so we predict that the vaccine will not alter the discriminative stimulus properties of fentanyl throughout the study and thus function as a negative control.

Experimental Design. *Discrimination Training:* Our laboratory has extensive experience utilizing drug discrimination procedures to study behavioral pharmacology of abused compounds [50,51]. Six adult rhesus monkeys (3 females and 3 males) will be trained to discriminate fentanyl from saline in their home cages. The behavioral task will be carried out on a touch screen monitor that is able to be mounted on the front of the monkey's home cage. Fentanyl will serve as the training drug at a dose of 0.01 mg/kg, which has been previously shown to function as a discriminative stimulus in both male and female rhesus monkeys [52,53]. During training sessions, fentanyl or saline will be administered intramuscularly, 15 minutes, prior to the start of the behavioral session. Once 15 minutes has elapsed, a red colored square and a green square will be displayed on the right and left side of the screen, respectively. A monkey must respond 30 consecutive times (Fixed Ratio 30) on the green colored square after a saline injection or on the red colored square after a fentanyl injection in order to earn a 1-gram banana flavored food pellet. Responses on the incorrect key reset the FR requirement on the appropriate key. A session terminates once a monkey earns 10 pellets or 5 minutes elapsed. Saline or fentanyl will be administered in a double-alternating fashion (FFSSF) such that the order of drug administration is effectively random. Behavioral sessions will be conducted 5-7 days a week. The training criteria will be: 1. ≥85% injection-appropriate responding prior to delivery of the first reinforcer, ≥90%injection-appropiate responding for the entire component, and response rates ≥0.1 responses/second [50,51]. Once a monkey has met the training criteria for 7 out of 8 consecutive training sessions, a multiple component training session will be enacted to mimic the cumulative dosing test procedures and will be made up of 5 components, identical to the single component training session. Accordingly, on a given day up to 5 components will be presented with fentanyl always being the last component tested (SF, SSF, SSSF, SSSSF, F). Once a monkey has met training criteria for 7 out of 8 consecutive sessions, testing procedures will begin.

Discrimination Testing: Test compounds will be administered in a cumulative dosing test procedure to determine the baseline substitution profile for the fentanyl training stimulus. Testing will be conducted twice a week on Tuesdays and Fridays, while fentanyl discrimination training will be conducted all other days of the week. Test sessions will be identical to training sessions, except that response requirement completion on either key will produce food and drug doses will be administered in a cumulative dosing procedure, made up of 5 components [54]. Each component will begin with a timeout period based on pretreatment time in which incremental doses of each drug (increase by 0.5 log units from one component to the next) are administered. We propose to determine a five-point cumulative dose function in a single session. Initial proposed dose ranges and pretreatment time is based on literature: fentanyl (0.001-0.032 mg/kg with a 15 min pretreatment time), heroin (0.032-0.32 mg/kg with a 5-minute pretreatment time), 6-monoacetylmorphine (6-AM) (0.01-1 mg/kg 5-minute pretreatment), and morphine (0.32-3.2 mg/kg 10-minute pretreatment), all administered intramuscularly [52-55]. Our experimental goal is to measure a broad dose range from inactive doses to doses that produce 100% fentanyl-appropriate responding or produce significant suppression in rates of operant responding.

We will initially determine naltrexone (positive control) effects on heroin, 6-AM and morphine's discriminative stimulus effects. Naltrexone (0.032 mg/kg), has been previously shown to significantly shift heroin, 6-AM, and morphine ED_{50} values [54,55] and will be administered 30 minutes before determination of a heroin, 6-AM or morphine cumulative dose-effect curve. Test drug doses of heroin, 6-AM, and morphine will be based on the previously established cumulative dosing curves and will be administered until maximum fentanyl-appropriate responding or marked suppression in rates of operant responding occurs. A negative control,

diazepam (1-10 mg/kg), will be tested, in order to determine the selectivity of the discriminative stimulus that has been trained. Previous studies have shown that diazepam does not substitute for mu opioid agonists [52].

Once each dose-effect function is established twice in a monkey, the monkeys will be vaccinated with a control vaccine composed of the tetanus toxoid, alum, and CpG ODN2006. Each monkey will receive three vaccinations, intramuscularly, spaced 2 weeks apart. Blood antibody levels will be measured after each vaccine administration in order to monitor titer levels, and this will also enable us to determine a time course of the vaccine's effects. During the vaccination interval, a monkey will resume normal discrimination training. After control vaccine administration, the test drug dose effect curves will be reestablished once following the cumulative dosing protocol. Finally, the heroin-vaccine will be administered and test drug dose effect curves will be redetermined once following the cumulative dosing protocol. The heroin-TT conjugated vaccine will consist of the heroin hapten conjugated to tetanus toxoid, alum, and CpG ODN2006. Prior to and after testing with heroin, blood levels will be drawn to monitor antibody levels.

Data Analysis: The primary dependent measures will be (1) percent fentanyl-appropriate responding (%FAR) {defined as (number of responses on the fentanyl-associated key divided by the total number of responses on both the fentanyl-and saline- associated keys) *100}, (2) injection appropriate responding prior to delivery of first reinforcer {defined as (injection-appropriate responses emitted before first reinforcer divided by total responses before first reinforcer)*100}, and (3) rates of responding {defined as (total responses emitted during response period divided by total time stimulus keys are displayed}. In addition, drugs that produce ≥90 percent fentanyl-appropriate responding will be considered to produce full substitution. %FAR and rates of responding will be analyzed by a two-way ANOVA with test drug dose and vaccination status as the main factors. A significant ANOVA will be followed by a Dunnett multiple comparison *post-hoc* test to compare vaccinated condition with control conditions. The criterion for significance will be set *a priori* at $p < 0.05$. An ED_{50} value for each monkey will also be calculated and will be defined as the test drug dose that produces 50% FAR. Log ED_{50} values will be calculated by log-linear interpolation and will be averaged to yield mean values and 95 percent confidence limits. Furthermore, dose-ratio analyses will be conducted to compare the potencies of heroin post-vaccination and after naltrexone to heroin effects in the warm water tail withdrawal procedure [16, 69].

Predicted Results: First, we expect heroin, 6-AM and morphine to substitute fully (≥90 percent fentanyl-appropriate responding) and the ED_{50} values will be ranked fentanyl>>heroin ≥ 6-AM >> morphine from most to least potent prior to and after control vaccine administration. Second, we expect the discriminative stimulus effects of the training drug fentanyl will not be altered following active heroin vaccine administration as evidenced by a lack of shift in the fentanyl ED_{50}. Lastly, after heroin-TT conjugate vaccine administration, we expect a rightward shift in heroin and 6-AM ED_{50}'s, but not morphine to produce fentanyl-like discriminative stimulus effects. It is predicted that the vaccine will produce a significant, >4-fold rightward shift in the ED_{50} of heroin and 6-AM to substitute for fentanyl. We expect to see the highest titer levels after the third injection of the heroin-TT conjugate vaccine while titer levels will drop after each heroin administration. These expected results would support further studies investigating this heroin-TT conjugate vaccine as a candidate immunopharmacotherapy for heroin abuse and addiction.

Specific Aim 2. Determine heroin-TT conjugate vaccine efficacy and selectivity on mu-opioid agonist antinociceptive effects in male and female monkeys.

Rationale This aim is to determine heroin-TT conjugate vaccine effects on heroin-induced thermal antinociception in a warmwater tail withdrawal procedure. Warmwater tail withdrawal procedures have been used in rhesus monkeys to determine if a test compound is able to block the noxious thermal stimuli produced by elevated water temperatures [59]. Previous studies have shown that mu opioid agonists are efficacious in blocking the tail withdrawal reflex in rhesus monkeys [16,63]. We propose to test heroin-TT conjugate vaccine effects on heroin-induced thermal antinociception for two reasons. First, this procedure will provide us with a spinally-mediated dependent measure to study the vaccine's effects and compare the results to the supraspinally, centrally-mediated effect studied in Aim 1's discrimination procedure. Second, antinociceptive effects of opioids post-immunopharmacotherapy administration is an especially clinically relevant property to investigate because it is common for individuals who are heroin dependent to experience hyperalgesia and a lack of response to opioid pain management [60-62,64].

Experimental Design: After discrimination studies have been completed, the same six moneys (3 FM and 3 M) will serve as the subjects in the warmwater tail withdrawal procedure. Heroin antibody titers will be collected prior to this experiment in order to ensure that monkeys have returned to a non-vaccinated status. For the entire study, all monkeys will be trained to sit calmly in restraint chairs with the tail hanging freely. The bottom ten centimeters

of each monkey's tail will be shaved and immersed in a thermal container of warm water. The time to remove tail (tail-withdrawal latency) will be measured at three different temperatures 38°C, 50°C, and 54°C with a stopwatch [16]. If a monkey fails to remove the tail at any temperature, a latency of 20 s will be assigned to that measurement. The temperature order will be presented randomly during each 15-minute component. In order to move on to testing experiments, monkeys must meet the following criteria: tail withdrawal from 38°C did not occur before the 20 s cutoff, and tail withdrawal occurred in ≤2 s from 50 and 54°C water.

During test sessions, drugs administered in a cumulative dosing procedure on Tuesdays and Fridays each week. The procedure will be comprised of five 15-minute sequential components. A single drug dose will be administered intramuscularly at the start of each 15-minute sequential cycle and each dose will increase in the next component by 0.5 log unit. After drug administration, tail withdrawal latencies will be redetermined. Initial proposed dose ranges are based on the published literature: heroin (0.032-1 mg/kg), fentanyl (0.0032-0.032 mg/kg), 6-AM (0.032-0.32 mg/kg), and morphine (0.32-10 mg/kg) [16,63-65,67]. Our experimental goal is to determine a broad dose range from inactive to fully active doses.

Second, a pretreatment protocol with naltrexone (0.032 mg/kg, 30 min) will be used as a positive control to assess shifts in heroin, 6-AM and morphine potency in warmwater tail withdrawal. Naltrexone (0.032 mg/kg), has been previously shown to significantly shift heroin, 6-AM, and morphine ED_{50} values in a warm water tail withdrawal procedure [66]. Test drug doses of heroin, 6-AM, and morphine will be based on the previously established cumulative dosing curves. Diazepam will serve as a negative control (1-10 mg/kg), which has previously been shown to have no antinociceptive effect in warmwater tail withdrawal procedures [87].

Once each dose-effect function is established twice in a monkey, the monkeys will first be vaccinated with a control vaccine and then the active vaccine as described above. Following each vaccination dosing schedule, antinociceptive dose-effect curves will be reestablished in each monkey using the same procedure previously described.

Data Analysis: Raw tail withdrawal latencies will be expressed as percentage maximum possible effect (%MPE) by using the equation %MPE = [(test latency − baseline latency)/(20 − baseline latency)] × 100, where test latency is the tail-withdrawal latency obtained after drug administration, baseline latency is the latency obtained at that temperature at the beginning of the session before drug administration, and 20 seconds will be the cutoff latency. In addition, ED_{50} values will be determined for each mu agonist before and after vaccination. ED_{50} values will be calculated by log-linear interpolation. Individual ED_{50} values will be averaged to yield mean ED_{50} and 95% confidence limits. The criterion for significance will be set *a priori* at the 95% level of confidence ($p < 0.05$). Furthermore, dose-ratio analyses will be conducted to compare the potencies of heroin post-vaccination and after naltrexone to heroin effects in the warm water tail withdrawal procedure [16, 69].

Predicted Results: Prior to and after control vaccine administration, we expect all mu-opioid agonists to produce dose-dependent antinociception. Furthermore, we expect the ED_{50}'s to be ranked as follows: fentanyl>heroin≥6-AM>>morphine from most to least potent. Second, after heroin-TT conjugate vaccine administration, we expect at least a 3.2-fold shift in the heroin and 6-AM ED_{50} values and no shift in the morphine or fentanyl ED_{50} value.

Specific Aim 3: Correlate heroin pharmacokinetics with behavioral effects.

Rationale. The goal of this aim is to correlate the pharmacokinetics of heroin and heroin's metabolites with the time-course of heroin's effects in a discrimination procedure and a warmwater tail withdrawal procedure. Heroin is rapidly metabolized into 6-AM (~2 mins). 6-AM penetrates the central nervous system more rapidly than heroin and it has been shown that most of heroin's behavioral effects are attributed to 6-AM [70,71]. Since the heroin-vaccine is designed to recognize the 6-acetyl group on heroin and 6-AM, it is important to understand vaccine effects on heroin biodistribution and pharmacokinetics.

Experimental Design: Six monkeys (3 M and 3 FM) will serve as subjects in this experiment. Blood samples will be collected after administration with the heroin control vaccine using a similar procedure to other studies our laboratory has conducted [51,68]. Monkeys will be placed in restraint chairs using the pole and collar technique. A temporary venous catheter with an injection port will be inserted into the saphenous vein and a baseline blood sample (2-3 mL) will be collected. Next, 0.32 mg/kg (IM) heroin will be administered and blood samples will be collected 3, 10, 30, 100, 180, and 300 minutes after administration. Blood samples will be transferred to Vacutainer© tubes, samples will be centrifuged at 1000 g for 10 minutes and then transferred to a labeled storage tube and frozen in a -80°C freezer until analysis. The blood will be analyzed for the presence of heroin, 6-AM, and morphine by high-performance liquid chromatography–tandem mass spectrometry. The VCU Pharmacology & Toxicology Department has a P30DA033934 center grant that supports this analysis and analysis will be under the guidance of Justin Poklis. After blood sample collection, the active heroin-vaccine will be administered and

samples will be recollected using the same technique. These blood withdrawal experiments will occur following each Specific Aim behavioral testing. Baseline heroin pharmacokinetics will be determined twice, once before each Specific Aim.

The same monkeys that served as subjects in Aim 1 and already trained to discriminate between 0.01 mg/kg fentanyl (IM) and saline in a two-key, food-reinforced discrimination procedure, will serve as subjects in the time-course determination of heroin's discriminative stimulus effects. The time-course test study will be the same procedure as the testing procedure in Aim 1 except 5-minute components will begin 3, 10, 30, 100, 180, and 300 minutes after a 0.32 mg/kg heroin injection [55]. This will be conducted after administration of the control and heroin-TT conjugate vaccine. Next, a heroin (0.32 mg/kg) time course will be examined in the warm-water tail withdrawal procedure using the same monkeys, and same procedure as Aim 2. A baseline tail withdrawal latency will be determined before drug treatment and 3, 10, 30, 100, 180, and 300 minutes after injection with 0.32 mg/kg heroin [58]. This procedure will be completed after vaccination with the control heroin vaccine, and again after injection with the heroin-TT conjugate vaccine.

Data Analysis: First, plasma heroin, 6-AM and morphine levels (nM) will be plotted as a function of time after administration of heroin. Data will be analyzed using a two-way ANOVA with test drug dose and control or active vaccine as the main factors. Holm-Sidak post hoc test will be used following a significant ANOVA to compare plasma levels at a given time point after drug administration [68]. For the correlation of the behavioral and pharmacokinetic data, the primary dependent measure in the discrimination time-course studies will be %FAR [see Aim 1 data analysis] and for the assay of thermal raw tail withdrawal latencies, %MPE [see Aim 2 data analysis]. A hysteresis analysis will be conducted using %FAR and %MPE as a function of plasma heroin levels (ng/ml), 6-AM, and morphine levels after heroin administration [51,68].

Expected Results: We do not expect a difference in plasma heroin levels due to the rapid conversion to 6-AM, the primary metabolite responsible for the centrally mediated effects of heroin. We expect plasma 6-AM levels to significantly increase at least 20-fold following heroin-TT conjugate vaccine administration. Furthermore, we expect this peripheral increase in 6-AM levels will correlate with a decrease in heroin efficacy to produce %FAR and %MPE in both behavioral assays. We expect no significant change in plasma morphine levels after vaccination. These results will support the hypothesis that the vaccine is sequestering heroin's behaviorally active metabolite to the periphery.

Potential problems & alternative outcomes

Based on our laboratories history of successfully training drugs as discriminative stimuli in rhesus monkeys and conducting warm water tail withdrawal, we anticipate no technical problems regarding the proposed experiments. Alternative outcomes may pose interpretive challenges that may require additional experiments. Two will be mentioned. One potential alternative outcome could be the heroin-TT conjugate vaccine is not effective or selective for heroin compared to other structurally dissimilar mu-opioid agonists. Although preliminary data suggest that the heroin-TT conjugate vaccine was not effective in shifting the oxycodone potency to decrease rates of operant responding in monkeys (Figure 2), whether the heroin-TT conjugate vaccine alters the behavioral pharmacology of fentanyl is an empirical question. This alternative outcome would suggest the heroin-TT conjugate vaccine is not recognizing the 6-acetyl group to produce the antibody response and future studies could determine the exact mechanism by which the heroin-TT conjugate vaccine is producing its antagonist effects. Furthermore, we will be able to compare potency shifts produced by the heroin-TT conjugate vaccine across a spectrum of mu-opioid agonists (heroin, 6-AM, morphine, and fentanyl) to further clarify the heroin-TT conjugate vaccine mechanisms. Another potential alternative outcome could be that sex differences emerge in either mu-opioid potency to produce discriminative stimulus or antinociceptive effects or in heroin-TT conjugate vaccine effects. This fellowship is not adequately powered to detect sex differences in the behavioral pharmacology of opioids alone and in the presence of a heroin-TT conjugate vaccine, and thus additional studies would be necessary to reproduce any potential sex differences noted. However, baseline differences in mu-opioid agonist potency would not preclude us from determining heroin-TT conjugate vaccine effects in male and female monkeys as a key experimental design feature in nonhuman primate research is the ability to conduct within-subject longitudinal experiments.

RESPECTIVE CONTRIBUTIONS

After joining Dr. Bank's (Sponsor) laboratory, I worked together with him to develop the ideas in this research proposal. The decision to utilize the two behavioral procedures in this project have been chosen based on my Sponsor's current funding. My committee members, Redacted by agreement and Steve Negus, PhD (Co-Sponsor) recommended the inclusion of Aim 3 to correlate pharmacokinetics of heroin with the two behavioral assays. The written content in the research proposal is my own work but includes the editing assistance of my sponsor and co-sponsor. Furthermore, I have met with my sponsor during the writing of this proposal and obtained advice on the art of grant writing. During my time in Dr. Banks lab, I have written the initial program for discrimination training and have begun training two monkeys on a two-key food-reinforced fentanyl vs. saline discrimination procedure. I have been presenting training data to my sponsor and cosponsor at meetings and obtained advice and feedback on proper training techniques. Redacted by agreement Redacted by agreement and a postdoc in my laboratory, Redacted by agreement have also provided advice on discrimination training techniques. Redacted by agreement has provided advice regarding the design and analysis of the pharmacokinetic experiments. Justin Poklis has offered his support for training me in using the mass spectrometry lab to quantify heroin and metabolites in blood samples. The preliminary Schedule Controlled Rate data included in the research proposal was conducted by Redacted by agreement a laboratory technician in the lab and the experiment was designed by Dr. Banks. The vaccine that we are testing in this proposal has been synthesized by Redacted by agreement at Scripps Research Institute and I have received his support to test the vaccine in the behavioral procedures. Several of my peers, whom have previously submitted proposals, have provided advice regarding the preparation of this proposal.

SELECTION OF SPONSOR AND INSTITUTION

I have been fascinated by drugs of abuse for most my life. This interest was reinforced during my undergraduate career at the University of Pittsburgh while majoring in Neuroscience. My first exposure to drugs of abuse while working as an undergraduate research technician in Dr. Charles Bradberry's self-administration laboratory was a defining point that helped me to transition to a career in research. I found it extremely interesting that nonhuman primates could be trained to self-administer drugs, and that laboratory models of drug self-administration could serve as a model for human drug-taking drug behavior. It was not until I was enrolled in my second year of pharmacy school at Virginia Commonwealth University School of Pharmacy that I realized I could pursue a career studying drugs of abuse and it seemed serendipitous that I was already enrolled at a top drug abuse research institution in the country.

The Department of Pharmacology and Toxicology at Virginia Commonwealth University has graduated over 300 Doctors of Philosophy. The department has over 35 faculty members with broad research interests in renal pharmacology, cellular pharmacology, behavioral pharmacology, testing of compounds for substance abuse disorders, inhalant pharmacology, neuroimmunology, drug development, genetics research, among many others. However, the long history of drug abuse research in the department is what particularly attracted me. The department also has a rich history of training students including one of the longest standing T32 training grants and provides many resources to help students succeed. In training students, the department emphasizes strong research skills, critical thinking and presentation of data at departmental seminars, all of which I believe will be a vital part of my training to become an independent researcher. The collaborative nature of the laboratories in the department is also something that has piqued my interest since working with other laboratories will introduce me to new techniques. Lastly, I choose this university is that it is one of the few universities that conducts nonhuman primate research. Since I have prior experience with nonhuman primates, I was aware of the advantages that nonhuman primates offer over other animal models in studying drugs of abuse, and I knew that I wanted my research to be conducted in nonhuman primates.

I selected my sponsor, Dr. Matthew Banks, because of the similarity in our pharmacy background and his success as a productive drug abuse researcher. He will serve as a very important resource as I pursue my second doctoral degree, since he has been through the same experience of pursuing two doctoral degrees. Although Dr. Banks has a clinical background, he has established himself as an assistant professor at the VCU Department of Pharmacology and Toxicology and has been conducting drug abuse research for 10+ years with nonhuman primates. Dr. Bank's own experience conducting drug abuse studies in nonhuman primates will provide me with the support to conduct the experiments proposed in this application. Furthermore, his nonhuman primate drug abuse laboratory possesses the equipment and resources needed to conduct my studies. Dr. Banks is extremely hard-working and intelligent and although I am his first student, he has been an excellent mentor to me for the past several years. With his leadership, I have been able to make a successful transition from the clinical field of pharmacy into basic science drug abuse research. Furthermore, he has guided me to think about science critically while also provided me the freedom to formulate my own ideas and work independently.

Dr. Steve Negus is a leading behavioral pharmacologist in the field of drug abuse and pain research. I selected him as a co-sponsor because he has extensive experience in mentoring graduate students and in the behavioral procedures and fields of pharmacology that I will be studying in this proposal. Furthermore, Dr. Negus is a very well respected scientist and I admire his ability to think deeply about data, try new experimental techniques, and write about science in a succinct, yet artistic manner. I have been fortunate to work with Dr. Negus in his Intracranial Self-Stimulation (ICSS) laboratory testing novel compounds in rodents, and the experience resulted in a foundational mentor-mentee relationship with Dr. Negus that will develop further throughout this project. Moreover, Dr. Negus possesses a rich history of conducting both drug discrimination and warmwater tail withdrawal procedures in nonhuman primates, both of which I will be studying in this application. Dr. Negus' current research on pain has exposed me to the pain field, and thus, he will serve as a key resource as I investigate a heroin vaccine's effects on antinociceptive properties of opioids.

RESPONSIBLE CONDUCT OF RESEARCH

Ongoing Responsible Conduct of Research Training

Virginia Commonwealth University (VCU) offers OVPR602: *Responsible Scientific Conduct* directed by the VCU Vice President for Research and Innovation, Francis L. Macrina. The 1-credit summer-long course I am enrolled in has 3 in-class sessions that include a 40-minute lecture by Dr. Macrina followed by 1-hour small group discussions of assigned case studies. Topics to be covered during the course include: the importance of proper record keeping, handling misconduct, data and intellectual property, conflict of interest, responsibilities of mentors and trainees, and authorship and collaboration. Cases and readings for the class are found within a textbook titled Scientific Integrity by F.L. Macrina. Case discussions have been especially beneficial in cementing my understanding of how to ethically assess and act in situations that may arise during my career. In addition to the in-class material, students are required to complete two online modules on human and animal research using the CITI (Collaborative Institutional Training Initiative) Program and American Association for Laboratory Animal Science (AALAS) Learning Library, respectively.

Along with the course I am currently enrolled in, I meet with my sponsor, Dr. Banks, formally, once a week, but more often two to three times a week to ensure my research is being carried out responsibly. Furthermore, my sponsor and cosponsor, Dr. Negus, hold biweekly joint lab meetings that I attend. Some period of the lab meeting is devoted to discussing animal health and wellbeing to ensure that our research subjects are being cared for humanely. Students are encouraged to present data and results and the interpretation of the results are discussed at length. Furthermore, we hold discussions twice a year that discuss the importance of scientific integrity and how to conduct animal research ethically and responsibly. Weekly lab meetings usually last one to two hours while my meeting with my sponsor is thirty to sixty minutes each week. In these meetings, we discuss ongoing projects and also how scientists should conduct experiments properly and responsibly. I also recently held a one 2-hour meeting with my committee members Redacted by agreement Redacted by agreement Dr. Matthew Banks and Dr. Steve Negus) but will hold future meetings every 6 months to discuss my experimental design and the development of the project.

Completed Responsible Conduct of Research Training

The Institutional Animal Care and Use Committee (IACUC) at VCU oversees and evaluates the proper care, use and humane treatment of animals used in research at VCU. Furthermore, IACUC also ensures the university is meeting federal guidelines for animal care used in scientific research and that researchers are following protocol throughout their research. Since I work very closely with animals, animal welfare is of extreme importance to me. I have completed a total of 10 CEU's in the format of online modules that covered working with nonhuman primates, rats and mice. Topics covered include interacting safely with animals, detecting pain/distress, administering injections, and minimizing pain and distress. I also completed a module titled "Working with the VCU IACUC" which discusses federal mandates related to animal research, avoiding unnecessary duplication in experiments, USDA Pain/Distress categories, proper surgical recovery analgesia, occupational health and safety, proper housing for nonhuman primates, the importance of enrichment and how to report misuse, mistreatment or noncompliance. Furthermore, IACUC visits our laboratory annually to conduct an inspection to ensure that our staff and research personnel are following the proper protocol for working with animals. IACUC holds live training sessions often that I plan to attend during my graduate career. Lastly, I attend a yearly 1-hour nonhuman primate refresher session held by Division of Animal Resources (DAR) Veterinarian Dr. Mario Dance. The training session revisits important safety and health aspects of working with nonhuman primates.

I recently completed 11 CEU's in the form of modules offered by the National Institute of Health (NIH) titled The Basic Science and the Biological Basis for Sex and Gender Related Differences and The Influence of Sex and Gender on Disease Expression and Treatment that address the importance of including females as subjects in experiments. The experiments that I am proposing to study in the grant have made for the inclusion of females as part of the NIH mandate. Moreover, I completed an online 1-credit course titled "Laboratory Safety" that was made up of modules regarding occupational exposure, radiation safety, chemical safety and fire safety. Since I work with controlled substances almost daily, I have also completed an online 1-hour module titled "Controlled Substances Training" that addressed the differences in scheduling of controlled substances, keeping up-to-date user logs, the proper handling/disposal of controlled substances and diversion of controlled substances.

SPONSOR AND CO-SPONSOR INFORMATION

The Sponsor (Dr. Matthew L. Banks) and Co-Sponsor (Dr. S. Stevens Negus) for this F31 application are faculty in the Department of Pharmacology at Virginia Commonwealth University. They collaborate on a grant related to the work proposed here, and the applicant would receive training in complementary skills that are already being used in multi-disciplinary research related to the applicant's career interests and goals.

(a) RESEARCH SUPPORT AVAILABLE

Sponsor: Dr. Matthew L. Banks

Dr. Banks has sufficient independent funding and research infrastructure to support this F31 application. Two of his grants (*marked below by an asterisk) are focused on topics closely related to this F31 application. A list of current funding is provided below:

*UH2DA041146 (Janda PI; Banks, Co-I) 9/15/15-7/31/17 $146,108
Immunopharmacotherapy for Mitigating Opioid Addiction
*R01DA037287 (Nicholson, Banks, Co-PI) 6/01/14-5/31/19 $81,813
Behavioral effects of NMDA antagonists/opioid agonist combinations
R01DA026946 (Negus, PI; Banks, Co-I) 7/01/09-6/30/20 $225,000
Medications Development for Stimulant Abuse
R01DA033364 (Lile, PI; Negus, Co-I) 2/1/13-12/31/16 $57,000
Medications Development for Cocaine: A Translational Approach in Monkey and Human

Although UH2DA041146 ends 7/17, we have already met the milestones proposed for the UH2 grant phase of the grant application and are anticipating the 3-year UH3 grant phase be awarded. In the event that the UH3 grant is not funded, there are sufficient resources from other funding sources listed above. In addition to these extramural resources, the sponsor also has startup funds available for the next several years to complete the experiments proposed in this fellowship application.

Co-Sponsor: Dr. S. Stevens Negus

R01DA026946 (Negus, PI) 7/01/09-6/30/20 $225,000
Medications Development for Stimulant Abuse
R01NS070715 (Negus, MPI; Sim-Selley, co-I) 10/1/09-7/31/19 $244,125
Neurobiology and Treatment of Pain
R01DA030404 (Negus and Sim-Selley; MPI) 7/1/11-4/30/16 $221,625
Endocannabinoid Modulation of Pain-Depressed Behavior
R01DA033930 (Negus; MPI) 4/14/12-3/31/17 $353,657
Synthetic Cathinones: A New Class of Illicit Drugs Affecting DAT & SERT
R01DA033364 (Lile, PI; Negus, Co-I) 2/1/13-12/31/16 $57,000
Medications Development for Cocaine: A Translational Approach in Monkey and Human
UH2DA041146 (Janda PI; Negus, Co-I) 9/15/15-7/31/17 $146,108
Immunopharmacotherapy for Mitigating Opioid Addiction

(b) PREVIOUS FELLOWS/TRAINEES

Sponsor: Dr. Matthew Banks

Dr. Banks is the primary mentor to 2 graduate students (both current) and 1 postdoctoral fellow (1 past, 0 current). Examples are below.

2013-2016 Blake Hutsell; Virginia Commonwealth University; Postdoctoral Fellow
Current Position: Assistant Professor in Psychology, East Carolina University

Co-Sponsor: Dr. Steve Negus

Dr. Negus has been the primary mentor to 11 graduate students (8 past, 3 current) and 13 postdoctoral fellows (11 past, 2 current), most of whom have gone on to careers in research. Recent examples of trainees are provided below. (*Received F award)

2008-10 Matthew Banks PharmD, PhD; Virginia Commonwealth University, Postdoctoral Fellow
Current Position: Assistant Professor, Department of Pharmacology and Toxicology, VCU
2010-13 *Andrew Kwilasz; Virginia Commonwealth University, PhD Pharmacology
Current Position: Postdoctoral Fellow, University of Colorado at Boulder
2010-13 *Clayton Bauer; Virginia Commonwealth University, MD/PhD Student
Current Position: Neurosurgery Resident, University of South Florida
2010-14 *Laurence Miller PhD; Virginia Commonwealth University, Postdoctoral Fellow
Current Position: Assistant Professor, Department of Psychology, Augusta University
2012-15 *Julie Bonano; Virginia Commonwealth University, MD/PhD Student
Current Position: Completing her last two years of medical school

(c) TRAINING PLAN, ENVIRONMENT, AND RESEARCH FACILITIES

The applicant is a PhD student who has earned her PharmD degree in 2015 and completed her first year of graduate school. The Training Plan is intended to structure supervision and mentoring in 7 general areas: (1) technical research skills in design, conduct, and interpretation of experiments, (2) academic instruction in areas related to the project, (3) writing and reviewing manuscripts, (4) management of lab operations, (5) ethics in research, (6) public speaking, and (7) translational research. Elements contributing to training goals in each area are described below, followed by information on Environment and Research Facilities.

Training Plan

(1) Technical Research Skills

To conduct studies proposed in this application, the applicant will require skills to (a) use drug discrimination procedures to evaluate the effects of a heroin hapten conjugate vaccine on heroin abuse-related subjective effects in male and female rhesus monkeys, (b) use high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) to conduct pharmacokinetic studies to elucidate the role of heroin and heroin metabolites in these behavioral dependent measures before and after heroin vaccine administration, and (c) use warm water tail withdrawal to compare heroin vaccine efficacy on a central nervous system abuse-related dependent measure (discrimination) with a spinally-mediated behavioral dependent measure (antinociception). Ms. Schwienteck acquired some behavioral experience with nonhuman primates and drug self-administration procedures with during her undergraduate studies and summer research rotation in my laboratory, but all other research skills will be new to the applicant.

Instruction on all behavioral procedures will be provided by the Sponsor (Dr. Banks) and members of the Sponsor's laboratory. Regarding drug discrimination, the applicant has learned the basic procedure and included her initial training results in the "Preliminary Data" section of her research strategy. Moreover, the female rhesus monkeys have recently been acquired and cleared quarantine and she has initiated the process of training the discrimination procedure in these female monkeys. Regarding the warm water tail withdrawal procedure, the applicant will learn the basic procedures once the monkeys are trained in the drug discrimination procedure.

Instruction on techniques for HPLC-MS analysis of heroin, heroin metabolite, and fentanyl plasma levels will be provided by Mr. Justin Poklis, a staff member in the VCU Department of Pharmacology and Toxicology and the member who runs the analytics core for the Department's Center on Drug Abuse Research P30DA033934. Mr. Poklis has trained other students in VCU in this procedure, and a letter from Mr. Poklis is appended to this application. These studies will also make use of the Department's Center on Drug Abuse Research P30DA033934 analytics core.

In addition to core training in these specific skills, the applicant will also receive training in related skills including drug preparation and delivery, maintenance and repair of equipment, experimental design, and data analysis. As the primary Sponsor, Dr. Banks will coordinate all technical training with the applicant with other faculty to assure satisfactory progress. In particular, the applicant will participate in twice-weekly lab meetings with Dr. Banks and other members from the Banks and Negus lab, and Dr. Banks will also meet individually at least weakly with the applicant. The Co-Sponsor, Dr. Negus, will meet monthly with the applicant regarding the behavioral studies. Dr. Negus's office is adjacent to the Sponsor's office and he maintains an open door policy with lab members, thus is available as needed for discussions with the applicant.

(2) Academic Instruction

The applicant is an outstanding student with a strong background of courses in basic sciences to complement her pharmacy school curriculum. Since starting her graduate work in September 2015, she has completed two 3-credit hour courses in Basic Concepts in Pharmacology (PHTX 630, the department's core course) and Behavioral Pharmacology (PHTX 633, which reviews basic principles of behavior and drug effects on behavior). To complement new directions in her research activities, Ms. Schwienteck will participate in additional academic training described below to increase her knowledge on topics pertinent to her research.

• *Courses offered at Virginia Commonwealth University*

(a) PHTX 691: Pain Pharmacology. This 3 credit hour course offered by the VCU Department of Pharmacology uses "Wall and Melzack's Textbook of Pain" together with selected readings to review basic principles on the measurement of pain in humans and research animals, the neurobiological mechanisms that underlie pain and nociception, and the effects produced in preclinical and clinical contexts by various drugs including opioids and non-steroidal anti-inflammatory drugs.

• *Webinars offered via the NIH Office of Research on Women's Health (http://orwh.od.nih.gov/)*

(a) Methods and Techniques for Integrating the Biological Variable Sex into Preclinical Research (see http://orwh.od.nih.gov/sexinscience/researchtrainingresources/methodstechniquesbiovar.asp). This is an ~8hr video of a 2014 Workshop on the emerging NIH mandate to include females in preclinical research, and this project is in part a response to this NIH mandate.

(b) The Science of Sex and Gender in Human Health online courses (see https://sexandgendercourse.od.nih.gov). This is a series of three 5-6 hr courses. Course titles are "The Basic Science and the Biological Basis for Sex- and Gender-Related Differences," "Sex and Gender Differences in Health and Behavior," and "The Influence of Sex and Gender on Disease Expression and Treatment." These courses will promote a translational perspective on implications of this project for human health.

• *Webinars offered via the Pain Research Forum (http://www.painresearchforum.org)*

The Pain Research Forum is an online resource for dissemination of information on current pain research. It includes highlights of recent publications, blogs for discussions on recent articles, and webinars offered approximately once per month that feature a core presentation by a leader in the field coupled with commentary by a group of 3-4 panelists. A library of 20 previous webinars is also available online and includes topics such as "The Use of Animal Models of Pain in Drug Discovery: Considerations and Challenges."

• *VCU Lab Meetings and Seminars*

(a) Banks Lab Meetings. The Banks lab meets twice weekly to discuss research progress by lab members and to review notable recent publications. Approximately half of lab research projects are focused on pain and analgesia research, with the other half on drug abuse research. During lab meetings, lab members present their own data, provide constructive criticism of data collected by their colleagues, and learn about research themes in areas that complement and extend their own research.

(b) Pain Interest Group. The Pain Interest Group is composed of VCU faculty and trainees with a broad interest in translational pain research. The group meets monthly and reviews current work by participant labs. This group provides one opportunity for the applicant to interact with other preclinical pain researchers in the VCU community including preclinical investigators Redacted by agreement

Redacted by agreement

(c) Departmental Seminars. The Pharmacology Department hosts weekly seminars with speakers from across the country. Recent seminars included "Neuroplasticity in the Brain Stress systems in Addiction" by Dr. George Koob of the National Institute on Alcohol Abuse and Alcoholism and "Sex Differences in Pain and Analgesic Responses" by Dr. Roger Fillingim of the University of Florida.

• *Attendance and Presentations at Scientific Conferences*

During the project period, the applicant and Sponsor or Co-Sponsor will attend one meeting of either the College on Problems of Drug Dependence (for exposure to a meeting focused on drug abuse research) and one meeting of the International Narcotics Research Conference (for exposure to a meeting focused on opioid pharmacology).

(3) Writing Manuscripts and Grants

Dr. Banks has published almost 70 peer-reviewed manuscripts in his relatively short scientific tenure and has a strong interest in writing. The Sponsor provides each training joining his laboratory with four books: *The Elements of Style* by Strunk and White, *On Writing Well* by William Zinsser, *Writing Science* by Joshua Schimel, and *How to Write A Lot* by Paul Silvia. Writing will proceed via periodic meetings and exchange of drafts for documents that include manuscripts and this grant application. Following writing examples, the

applicant and the sponsor will work through writing examples from published texts described above. In addition, Dr. Banks will also begin to provide training on manuscript reviews for scientific journals. Additional writing assistance is available through the VCU Writing Center and a graduate level 2-credit-hour course on Scientific Writing and Grantsmanship offered through the VCU Department of Anatomy (ANAT 620).

(4) Management of Lab Operations

Training in lab management includes several components. First, all graduate students are trained in lab safety; basics of animal use and husbandry; proper use, maintenance and repair of equipment; management of supplies to include proper documentation for use of schedule drugs; and proper use of radioactive materials. Second, to gain skills and experience in personnel management, advanced students who have mastered essential techniques will receive opportunities to train and potentially manage activities of technical staff or junior students. Third, training in management of interactions with regulatory groups (e.g. by the IACUC for use of animals and DEA for use of scheduled drugs) will be accomplished by integrating the applicant into development of lab protocols, maintenance of regulatory documentation, and preparation for inspections. Lastly, training in budget management will be accomplished by introducing the applicant to budget worksheets for lab projects and working with the applicant in managing the budget of her F31 if awarded.

(5) Ethics in Research

Training in the responsible conduct of research is provided in two venues. First, the VCU graduate school offers a course entitled “Scientific Integrity.” This course is directed by Dr. Francis Macrina, Vice President of Research at VCU and a noted authority on scientific ethics. The course meets NIH training requirements on responsible conduct of research. Second, continuous training in responsible conduct of research is provided in the context of the Sponsor’s laboratory meetings. Topics related to the responsible conduct of research are addressed quarterly.

(6) Public Speaking

Every semester, all students in the VCU Department of Pharmacology take a 1 credit hour course titled Pharmacology Research Seminar (PHTX 690), which provides instruction on preparation of slide sets, oral delivery, and response to questions. Each week, a different student delivers a seminar on his or her research to the entire department, and over the course of the academic year, all students give a seminar. Each student’s seminar is evaluated by three faculty members who critique the presentation for style and content of slides and oral delivery. Students are also required to ask questions during seminars by other students. Additional opportunities to hone public speaking skills are provided in twice-weekly lab meetings. Lastly, opportunities for public speaking are provided via participation at scientific meetings and in an annual lab exchanges between the Banks lab and labs at other institutions (e.g. Wake Forest, Emory University, and University of Kentucky).

(7) Translational Research

Training will be provided in translational research skills by the Sponsor by the applicant’s clinical mentor and dissertation committee member, Redacted by agreement

Environment

Ms. Schwienteck will conduct her research in an environment with a history of expertise and productivity in preclinical research on pain, analgesia and drug abuse. The Sponsor (Dr. Banks), the Co-Sponsor (Dr. Negus) and the members of their laboratories will provide the most proximal layer of support and collegial interactions for Ms. Schwienteck. The Sponsor has conducted drug addiction research for over 10 years and served as faculty member on NIDA training grants for more than 3 years. At present, the Sponsor’s laboratory includes two graduate students including Ms. Schwienteck. The Co-Sponsor has conducted pain/analgesia research for 25 years, served as faculty member on NIDA training grants for more than 20 years, and trained more than 20 graduate students and postdoctoral fellows, most of whom have gone on to careers in academic or industry research. At present, the Co-sponsor’s laboratory includes a junior faculty member, two postdoctoral fellows, and three graduate students.

A second layer of support in the candidate’s environment is provided by the Department of Pharmacology, which has a 45-year record of research in areas that include drug abuse, pain, and analgesia, and which currently hosts multiple faculty members with NIH support to conduct research in these areas. The Department is chaired by Dr. Bill Dewey, a NIDA-funded researcher with expertise in opioid pharmacology and PI on one of the longest running training grants in the NIDA portfolio.

A final layer of support is provided by colleagues at institutions outside VCU. In particular, the Sponsor and Co-Sponsor collaborate with chemists Redacted by agreement Redacted by agreement and neurobiologists Redacted by agreement Redacted by agreement with an interest in research on mechanisms and treatments for drug addiction. Additionally, Dr. Banks participates in an annual laboratory exchange with colleagues at other institutions (e.g. Redacted by agreement Redacted by agreement These exchanges provide opportunities for Banks-lab trainees to present their data in a supportive environment and meet colleagues at other universities.

Research Facilities

The Sponsor occupies an Redacted by agreement laboratory that was renovated in 2007-2008 and located within the AAALAC-accredited Redacted by agreement n the Redacted by agreement These Reda rooms include (1) a housing room equipped with 16 custom cages for studies of drug- and food-maintained responding, (2) an adjacent procedure room equipped with a surgical table and surgical light to be used for minor health-maintenance and catheter-maintenance procedures in drug self-administration monkeys, (3) a housing room equipped with 12 custom cages for operant studies of drug discrimination, (4) an adjacent procedure room, (5) a housing room equipped with 12 holding cages for monkeys not involved in operant studies, and (6-7) two "control" rooms that house computer control equipment, administrative desk space for technical staff, work space for drug preparation and equipment maintenance, and storage space for supplies. All rooms are temperature and humidity controlled and equipped with programmable lighting for controlled light-dark cycles. All housing rooms are equipped with automatic water-delivery systems.

Six (6) cages for drug- and food-maintained responding (Redacted by agreement would be dedicated to studies proposed in this application. These cages are currently available and can be allocated immediately. Each cage has a custom front door designed to accommodate attachment of a detachable, custom-built operant touchscreen panel. Each cage is also equipped with a pellet dispenser (ENV-203-1000; Med Associates, St. Albans, VT), and custom programs are written in ABET notation programmed on the Whiskers server. Operant panels, feeders and syringe pumps are controlled by custom software run on PC computers and interface equipment. Backup cages, operant panels, pellet dispensers and syringe pumps are also already in our inventory. In addition to these core laboratory spaces, other general use "peripheral" laboratory resources are also available for the conduct of the proposed studies. These peripheral resources include the following: (a) a surgical suite for conduct of IV catheterization surgeries in rhesus monkeys, (b) steam and gas sterilizers for sterilization of surgical instruments and supplies, (c) cage wash facilities for biweekly cage washing, (d) walk-in refrigerators for storage of food and fruit, (e) shop facilities for design, manufacture and/or maintenance of equipment, and (f) a Mettler balance and weighing table for use in weighing out drugs. Office space for trainees is located in the same building and equipped with wireless internet access and cubicles for up to four graduate students. Office space for the Sponsor, Co-Sponsor and trainees is located on the same floor, so Ms. Schwienteck will have ready access to the Sponsor, Co-Sponsor and senior staff throughout her training. In addition, a 300-square-foot conference room located on the same floor as the office space is available for laboratory meetings and as study/writing space for the applicant and the other graduate students. This design of administrative space consolidates activities by members of the Sponsor's laboratory and encourages interactions between lab members.

(d) NUMBER OF FELLOWS/TRAINEES TO BE SUPERVISED DURING THE FELLOWSHIP

The Sponsor (Dr. Banks) current has two predoctoral PhD trainees and no postdoctoral trainees. The Sponsor anticipates recruiting a postdoctoral trainee during the term of the proposed fellowship. The Co-Sponsor (Dr. Negus) currently has three predoctoral trainees and two postdoctoral trainees. The three predoctoral trainees include two MD/PhD students, and one PhD student.

(e) APPLICANT'S QUALIFICATIONS AND POTENTIAL FOR A RESEARCH CAREER

Sponsor's Statement:

Redacted by agreement

Redacted by agreement

Co-Sponsor's Statement:

Redacted by agreement

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Withheld pursuant to exemption

Redacted by agreement

of the Freedom of Information and Privacy Act

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING

The Department of Pharmacology and Toxicology has over 35 full-time faculty, 33 PhD and 4 MS students, and 20 Postdoctoral Fellows. The Department is currently ranked in the top 10 NIH–funded Pharmacology and Toxicology programs in the country, is the largest graduate program in the School of Medicine and has graduated over 310 PhD scientists over the last 50 years.

The Department of Pharmacology and Toxicology at Virginia Commonwealth University has employed strong leadership and superior teaching since 1953. Alumni of the department have achieved prominent roles in academia, industry and government. The goal of the department is to improve the treatment of medical disorders through a better understanding of the pharmacology of agents and their mechanisms of action with the purpose of developing safer and more effective pharmacological therapies to enhance human health. The department is committed to excellence in research, teaching and service in the area of pharmacology and toxicology, and in the training of graduate students to become independent, highly regarded scientists and expert teachers in pharmacology.

The major component of the degree program is conducting an original independent research project under the supervision of a faculty member. Although the program prepares students for a research oriented career, there is flexibility in allowing for alternative careers in biomedical sciences. This includes availability of advanced elective courses in careers in biomedical sciences which exposes students to speakers from various industries. Additionally, there is a dedicated career counsellor on the medical campus to help students in CV preparation, etc. Each student is also required to initiate an Independent Development Plan (IDP) and report it to the Graduate Program Director.

Currently, 19 members of the department concentrate their research and teaching efforts in the area of drugs of abuse. The faculty expertise available to Kathryn Schwienteck include her mentor, D.r Matt Banks, Dr. Steve Negus, Redacted by agreement who are internationally recognized behavioral pharmacologists. In addition to these, Redacted by agreement have expertise in the cellular aspects of drug abuse. The department also holds monthly drug abuse research seminars and many visiting seminar speakers in the Department are prominent researchers in this area. The department has maintained a Training Grant in Drug Abuse, now in its 40th year. Thus historically the department has a strong commitment to the area of drug abuse and continues to maintain a vibrant environment for the training of graduate students.

VERTEBRATE ANIMALS

1. Provide a concise description of the proposed procedures to be used that involve vertebrate animals. Identify the species, strains, ages, sex and total number of animals by species to be used. If dogs or cats are proposed, provide the source of the animals.

This application proposes to use adult male and female rhesus monkeys (Macaca mulatta) as research subjects. Animal maintenance and research are conducted in accordance with the 8th edition guidelines for the care and use of animals in biomedical research [73]. Animals are housed in temperature- and humidity-controlled housing rooms, and a 12 h light-dark cycle is in effect (lights on from 6 a.m. to 6 p.m.). The facility is licensed by the United States Department of Agriculture and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and research protocols are approved by The Institutional Animal Care and Use Committee (IACUC).

All experiments will be conducted with the same 6 monkeys (3 females, 3 males). Monkeys will be individually housed in stainless steel chambers Redacted by agreement Redacted by agreement Each chamber is equipped with a perch and squeeze bars. Monkeys are housed individually to assure that each touchscreen monitor can be accessed by only one monkey and to protect drug-exposed animals from antagonistic interactions with other monkeys. All monkeys will be maintained on a diet of high fiber food biscuits (Purina, St. Louis, MO), fresh fruit and novel foods will be provided as enrichment in foraging devices. In addition, monkeys can receive up to 50 1-gm banana-flavored food pellets (Purina, St. Louis, MO) during daily operant sessions. Food deprivation will not be required for the proposed studies, although biscuit delivery will be restricted to after the session. Monkeys will have visual, auditory and olfactory contact with each other and with researchers throughout the study. Operant procedures, foraging toys, and music/videos played in housing rooms will provide an opportunity for environmental manipulation and enrichment. Additionally, monkeys will be able to spend certain weekends in a playpen for supplemental enrichment. Technical and veterinary staff will monitor the health of the animals multiple times daily. Since monkeys will be administered a novel vaccine during this study, blood samples will be collected monthly for diagnostic evaluations of complete blood counts and blood chemistries. Monkey's weights will also be monitored throughout the studies.

Specific Aim 1: Monkeys will be trained to respond on two-key food-reinforced fentanyl vs. saline discrimination procedure. Monkeys will receive cumulative dosing injections of mu agonists over a 1-2 hour test session. One important consideration in the proposed studies is the risk of drug-induced toxicity, and several procedural steps will be undertaken to mitigate this risk. First, rates of food-maintained responding will be used as a measure of drug-induced toxicity because of its high sensitivity to drug effects [16,79,80]. Opioids routinely decrease rates of food-maintained responding at doses below those that produce signs of behavioral or physiological toxicity, and thus food-maintained rates of responding will be closely monitored during cumulative dosing procedures. Second, video monitors of each monkey's cage allow for real time observations of monkey's behavior during operant procedures. Third, technical and veterinary staff will be trained to anticipate potential toxicity and will monitor monkeys multiple times daily. Respiratory depression and sedation constitute one class of toxic effects, and naltrexone will be maintained in the animal housing room to permit rapid treatment of respiratory depression and sedation should they occur.

Specific Aim 2: Monkeys are fitted with a stainless steel metal collar that will enable the researcher to pole train the monkeys to be removed from the cages and placed in restraint chairs for tail withdrawal procedure, blood draws or cage changes. Monkeys are positively reinforced with treats for sitting calmly in their chair during which their tails will be dipped in water heated to three different water temperatures (38°C, 50°C, and 54°C). During tail withdrawal experiments, monkeys will be chairs for no more than three hours.

Specific Aim 3: Monkeys will be placed in restraint chairs for blood collection 3, 10, 30, 100, 180 and 300 minutes after drug injection. Approximately 2-3 mL of blood will be drawn at each time point. This will not occur more than once every two weeks in order to minimize blood loss. In addition, hematocrit levels will be determined before all blood collection experiments.

2. Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, in vitro).

We propose to use animals for the proposed studies to permit systematic, precise and extensive manipulation of independent variables and long-term, within-subject monitoring of dependent variables that would not be possible in studies with humans. Studies in animals permit precise control over both (1) the nature of the food reinforcer and the contingencies that govern their availability, and (2) other aspects of the environment (e.g. other food and drug sources) that might influence behavioral tasks.

Male and female rhesus monkeys are proposed as the primary species for two general reasons. First, the close phylogenetic relationship to humans continues to make rhesus monkeys excellent models for research on many facets of human disease, including drug abuse and vaccine development [74, 75, 76, 81]. In this application, we are especially concerned with the phylogenetic similarities between humans and rhesus monkeys for studies of pharmacodynamics, pharmacokinetics and immune responses to vaccines. In regards to pharmacodynamics, the dopaminergic and opioidergic systems are more similar between humans and rhesus monkeys than between humans and rats and this appears to influence the pharmacology of drugs like those proposed for study in this application [74,77,78]. Nonhuman primates have been used to study the immunogenicity of vaccines over many decades for diseases such as polio, HIV, and Hepatitis B/C due to their similar immunological response and antibody kinetic profile and therefore, are prime candidates to study the efficacy of a heroin vaccine [82,83].

A second rationale for proposing use of nonhuman primates relates to logistical advantages for the conduct of the proposed studies. In particular, this application proposes within-subject evaluation of effects produced by a novel heroin-vaccine on two sophisticated measures of behavioral performance. Within-subject studies are proposed to maximize statistical and interpretive power and minimize animal use. Training time for a discrimination procedure can take up to 6 months, and once monkeys are trained, the discrimination may be maintained as long as the animals are in good health.

3. Describe the interventions to minimize discomfort, distress, pain and injury. These include analgesia, anesthesia, sedation, palliative care and humane endpoints.

Monkeys are fitted with a stainless steel metal collar that will enable the researcher to pole train the monkeys to be removed from the cages and placed in restraint chairs for tail withdrawal procedure, blood draws or cage changes. Monkeys are positively reinforced with treats for sitting calmly in their chair for blood collection. During cage changes, monkeys will sit in their chair for approximately a half hour, while during tail withdrawal and blood drawl, monkeys will not be in the chairs for more than three hours. If ketamine is required for health checks, approximately 3-5 mg/kg is administered intramuscularly.

4. Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.

Euthanasia is not a routine or planned part of the proposed research. In the event that an animal's health status dictates euthanasia for humane considerations, veterinary staff will perform euthanasia by slow infusion of intravenous pentobarbital, a procedure consistent with recommendations by the panel on euthanasia of the American Veterinary Medical Association.