RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 09/15/2020

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Grant Number: 1R01AA029023-01
FAIN: R01AA029023

Principal Investigator(s):
Donna M Platt, PHD

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

Joshua Clark
2500 N State Street

Jackson, MS 392164505

Award e-mailed to: ORSPpostaward@umc.edu

Period Of Performance:
Budget Period: 09/15/2020 – 07/31/2021
Project Period: 09/15/2020 – 07/31/2025

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $471,208 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSISSIPPI MED CTR in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 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 Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number R01AA029023. 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,

Jeffrey Thurston
Grants Management Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Additional information follows

SECTION I – AWARD DATA – 1R01AA029023-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$127,906
Fringe Benefits	$35,303
Personnel Costs (Subtotal)	$163,209
Consultant Services	$4,050
Materials & Supplies	$13,500
Travel	$3,600
Other	$82,061
Subawards/Consortium/Contractual Costs	$44,507
Federal Direct Costs	$310,927
Federal F&A Costs	$160,281
Approved Budget	$471,208
Total Amount of Federal Funds Obligated (Federal Share)	$471,208
TOTAL FEDERAL AWARD AMOUNT	$471,208
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$471,208

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$471,208	$471,208
2	$443,607	$443,607
3	$510,067	$510,067
4	$472,924	$472,924
5	$466,274	$466,274

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

Fiscal Information:

CFDA Name:	Alcohol Research Programs
CFDA Number:	93.273
EIN:	1646008520A2
Document Number:	RAA029023A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023	2024
AA	8470415	$471,208	$443,607	$510,067	$472,924	$466,274

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

NIH Administrative Data:
PCC: AN E / **OC**: 41021 / **Released**: eRA Commons 09/15/2020
Award Processed: 09/16/2020 12:09:37 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AA029023-01

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 – 1R01AA029023-01

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).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

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) R01AA029023. 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/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements

and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AA Special Terms and Conditions – 1R01AA029023-01

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

Based on a review of your application and the need to effect NIAAA budgetary and programmatic goals, your requested direct cost funding has been adjusted.

This award is issued in accordance with NIH Fiscal Policies in effect for FY 2020 (see NIH Guide Notice NOT-OD-20-068).

SALARY LIMITATION: None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Therefore, this award and/or future years are adjusted accordingly, if applicable.

Current salary cap levels can be found at the following URL:
http://grants1.nih.gov/grants/policy/salcap_summary.htm.

OTHER LEGISLATIVE MANDATES: Other statutory requirements are described in NIH Guide Notice NOT-OD-20-066.

CONSORTIA: This award includes funds awarded for consortium activity with Redacted by agreement Redacted by agreement Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS).

INFORMATION: In order to redistribute awards more evenly throughout the year, budget periods are being adjusted. This award is issued with a **shortened initial budget period** and with 12 months of support. Continuation awards will cycle each year on **August 1.**The noncompeting continuation Research Performance Progress Report (RPPR) is due the 15th of the month preceding the month in which the budget period ends.

INFORMATION: The recycling of this award has changed the receipt date for the next competing continuation (type 2) application if applicable. Consult "application receipt, review and award schedule" in the NIH Grants Policy Statement (http://grants.nih.gov/grants/policy/policy.htm#gps) for the established deadline date.

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: Celia B. Herlihy
Email: celia.herlihy@nih.gov **Phone**: 301.443.4705

Program Official: Mark Egli
Email: megli@mail.nih.gov **Phone**: 301-594-6382 **Fax**: 301-594-0673

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AA029023-01

INSTITUTION: UNIVERSITY OF MISSISSIPPI MED CTR

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$127,906	$127,906	$127,906	$127,906	$127,906
Fringe Benefits	$35,303	$35,303	$35,303	$35,303	$35,303
Personnel Costs (Subtotal)	$163,209	$163,209	$163,209	$163,209	$163,209
Consultant Services	$4,050	$4,050	$4,050	$4,050	$4,050
Materials & Supplies	$13,500	$13,500	$13,500	$13,500	$13,500
Travel	$3,600	$3,600	$3,600	$3,600	$3,600
Other	$82,061	$73,125	$116,002	$92,039	$87,749
Subawards/Consortium/Contractual Costs	$44,507	$44,507	$44,507	$44,507	$44,507
TOTAL FEDERAL DC	$310,927	$301,991	$344,868	$320,905	$316,615
TOTAL FEDERAL F&A	$160,281	$141,616	$165,199	$152,019	$149,659
TOTAL COST	$471,208	$443,607	$510,067	$472,924	$466,274

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	55%	55%	55%	55%	55%
F&A Cost Base 1	$291,420	$257,484	$300,361	$276,398	$272,108
F&A Costs 1	$160,281	$141,616	$165,199	$152,019	$149,659

PI: **Platt, Donna M**	Title: GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol	
Received: 04/28/2020	FOA: PA19-056 Clinical Trial:Not Allowed	Council: 10/2020
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 AA029023-01	Dual: MH	Accession Number: 4430655
IPF: 5390304	Organization: UNIVERSITY OF MISSISSIPPI MED CTR	
Former Number:	Department:	
IRG/SRG: NAL	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 331,430 Year 2: 322,886 Year 3: 371,975 Year 4: 346,790 Year 5: 342,053	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Donna Platt	UNIVERSITY OF MISSISSIPPI MED CTR	PD/PI
Redacted by agreement	University of Mississippi Medical Center	Post Doctoral
	Redacted by agreement	Co-Investigator
	University of Mississippi Medical Center	Co-Investigator
	University of Mississippi Medical Center	Co-Investigator

OMB Number: 4040-0010
Expiration Date: 12/31/2022

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**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION
Organizational DUNS*: 9288244730000

Legal Name*: UNIVERSITY OF MISSISSIPPI MED CTR
Department:
Division:
Street1*: 2500 N STATE STREET
Street2:
City*: JACKSON
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 392164505

Person to be contacted on matters involving this application
Prefix: First Name*: Joshua Middle Name: Last Name*: Clark Suffix:
Position/Title:
Street1*: 2500 N State Street
Street2:
City*: Jackson
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 39216-4505
Phone Number*: 601-815-5000 Fax Number: Email: sponsoredprograms@umc.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 646008520

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

12. PROPOSED PROJECT
Start Date* Ending Date*
09/01/2020 08/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MS-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Donna Middle Name: M Last Name*: Platt Suffix:
Position/Title: Professor
Organization Name*: UNIVERSITY OF MISSISSIPPI MED CTR
Department:
Division:
Street1*: 2500 North State Street
Street2:
City*: Jackson
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 39216-4505
Phone Number*: 601 984 5896 Fax Number: Email*: dplatt@umc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,645,048.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,645,048.00
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*: Joshua Middle Name: Last Name*: Clark Suffix:
Position/Title*: Director, Sponsored Programs, Pre-Award
Organization Name*: University of Mississippi Medical Center
Department:
Division:
Street1*: 2500 N State Street
Street2:
City*: Jackson
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 39216-4505
Phone Number*: 601-815-5000 Fax Number: Email*: fclerk@umc.edu

Signature of Authorized Representative*
Felicia Clerk

Date Signed*
04/28/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Platt_cover_letter_-_Feb_2020_-_edited.pdf

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

OMB Number: 4040-0010
Expiration Date: 10/31/2019

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: UNIVERSITY OF MISSISSIPPI MED CTR
Duns Number: 9288244730000
Street1*: 2500 N STATE STREET
Street2:
City*: JACKSON
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 392164505
Project/Performance Site Congressional District*: MS-003

Project/Performance Site Location 1

❍ 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:
DUNS Number:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site

Redacted by agreement

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

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 _ 7 _ 8

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:

Animal Welfare Assurance Number D16-00174

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*	Platt_summary_-_Feb_2020_-_edited.pdf
8. Project Narrative*	Platt_narrative_-_Feb_2020.pdf
9. Bibliography & References Cited	Platt_lit_cited_-_Feb_2020_-_edited.pdf
10.Facilities & Other Resources	Platt_facilities_-_Feb_2020_-_edited.pdf
11.Equipment	Platt_equipment_-_Feb_2020.pdf

Funding Opportunity Number: PA-19-056. Received Date: 2020-04-28T12:18:24.000-04:00

The abuse of alcohol is controlled by multiple effects of the drug, including its subjective, reinforcing, and relapse-inducing effects. Preclinical methods have been developed to assess the contribution of these controlling factors and their neurobiological underpinnings, and to provide empirically based models for evaluating potential treatment strategies. Alcohol's ability to potentiate the activity of γ-aminobutyric acid (GABA) at $GABA_A$ receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both humans and laboratory animals, making this system an attractive candidate for the development of therapeutics. The complex molecular biology of $GABA_A$ receptors raises the possibility that subtype-selective agents might be developed with therapeutic specificity against alcohol. In this application, we will investigate the role of γ- and δ-containing α4$GABA_A$ and α6$GABA_A$ receptor mechanisms in nonhuman primate and rodent models of the abuse-related effects of alcohol. We will use first-in-kind compounds that are selective for α4δ, α6δ, α4γ, and/or α6γ$GABA_A$ receptors to investigate the contribution of these subtypes to: 1) the discriminative stimulus effects of alcohol in monkeys trained to discriminate intra-gastrically-administered alcohol from vehicle, 2) the reinforcing effects of alcohol in monkeys orally self-administering alcohol, and 3) the relapse-inducing effects of alcohol in rats trained in either cue-induced reinstatement or alcohol deprivation effect procedures (**Specific Aim 1**). Understanding the neuropharmacological mechanisms underlying the addictive effects of alcohol is an important initial step in the development of candidate pharmacotherapies for the treatment of alcohol abuse and dependence. The degree to which the effects of γ- and δ-selective α4$GABA_A$ and α6$GABA_A$ ligands selectively modify alcohol-controlled behavior will be evaluated in monkeys that self-administer a sucrose solution instead of alcohol and in rats trained in a cue-induced sucrose seeking procedure. In monkeys, concurrent observational studies will characterize the effects of the ligands, alone or combined with alcohol, on unconditioned motor behavior (**Specific Aim 2**). The ability of these ligands to mimic or modulate the discriminative stimulus effects of alcohol, alcohol self-administration, and cue-induced alcohol seeking and relapse-like drinking at doses that do not produce a generalized disruption of behavior or debilitating side effects may be predictive of potential therapeutic utility. Finally, we will investigate the utility of selective GABAergic ligands with favorable side effect profiles to serve as co-therapies in a model of medication-assisted treatment (**Specific Aim 3**). These studies will make use of a novel resurgence model of contingency management developed recently in our laboratory and, initially, ligands that either mimic or attenuate the behavioral effects of alcohol. Integration of results from the aims will continue to yield needed information about neuropharmacological mechanisms underlying the addictive effects of alcohol and begin to identify clinical scenarios in which pharmacological approaches might be expected to produce improved patient outcomes.

Alcohol's ability to enhance the activity of γ-aminobutyric acid (GABA) at $GABA_A$ receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in humans, making this system an attractive candidate for the development of therapeutics. The complex molecular biology of $GABA_A$ receptors raises the possibility that subtype-selective drugs can be developed with therapeutic specificity against alcohol use disorders. Our studies will yield needed information about neuropharmacological mechanisms underlying the addictive effects of alcohol and begin to identify clinical scenarios in which specific pharmacological approaches might be expected to produce improved patient outcomes.

FACILITIES AND OTHER RESOURCES: UMMC

Institutional support: The PI is a tenured Professor in the Departments of Psychiatry & Human Behavior and Neurobiology & Anatomical Sciences at the University of Mississippi Medical Center (UMMC) in Jackson, the state capital. UMMC is the only academic medical center in Mississippi, with a tripartite mission of research, education, and patient care. Dr. Platt was recruited in 2013 to help facilitate expansion of research, education, and patient care in substance use disorders within the Department of Psychiatry & Human Behavior. At present, ~30% of NIH-funded neuroscience-related grants at UMMC are from NIDA, NIAAA, and SAMHSA. In addition to dedication to substance abuse research, UMMC has shown a strong commitment to laboratory animal-based research, including nonhuman primate (NHP) research. UMMC supports a colony of approximately 100 macaques and has room for growth. At present, there are eight faculty-level NHP researchers at UMMC. Dr. Platt's faculty position is referred to as "Research Track", with the expectation of 75% research, 15% administrative, and 10% teaching effort. Dr. Platt is the Director of the Division of Neurobiology and Behavior Research (8/2018 – present) within the Department of Psychiatry & Human Behavior.

Laboratory: The nonhuman primate research described in this proposal will be conducted in Dr. Platt's space in Redacted by agreement Three rooms (approximately 1500 ft^2 of newly renovated space) have been designated for Dr. Platt's NHP research. Two rooms are housing areas for monkeys. The other room contains the computer-control systems.

The rodent research described in this proposal will be conducted in Dr. Platt's behavioral suite in Redacted by Redacted by agreement Three rooms have been designated for Dr. Platt's rodent endeavors. One room is a housing room for rats. The other two rooms are dedicated for self-administration/reinstatement testing and contain the operant conditioning chambers needed for the proposed research.

Center for Comparative Research: The animal research enterprise at UMMC is overseen and augmented through the Center for Comparative Research (CCR). A full-time veterinary medicine staff is available for medical consultation and assistance; supervised technicians are responsible for daily care of animals. Redacted by agreement are treatment areas and surgery suites. The facility is outfitted with anesthetic machines with state-of-the-art monitoring equipment. Isolation and quarantine facilities are available. The CCR Program is fully AAALAC accredited, and offers frequent training on- and off-site in animal care, use, and regulatory compliance.

Computer: Behavioral studies are controlled by Med Associates software, interfaces, and IBM computers that are available within the laboratory. In addition, computers are available to all personnel for word processing, data analysis and graphics. Several laser printers are available as well. The laboratory has full access to the UMMC information technology group, along with internet and LAN access via Ethernet. All experimental data are stored in designated areas on a server, which is automatically backed up daily and located outside of the UMMC campus.

Office: Office space is available for Dr. Platt and all personnel involved in the behavioral studies. Within the Department of Psychiatry & Human Behavior, there are administrative and clerical personnel, facilities and equipment for grant administration. In addition, there is storage space for record keeping.

Other: A well-equipped machine and electronics shop is available at UMMC. A separate "wet lab" space, shared by NHP researchers, is available for compound preparation, equipped with necessary equipment (e.g., safes, precision balance, pH meters, Analox AM1 series analyzer, etc.). The resources of various UMMC Core Facilities and Centers also are available, including the Center for Psychiatric Neuroscience, Center for Biostatistics and Bioinformatics, and the Rowland Medical Library.

Intellectual Resources: Dr. Platt and her laboratory staff are located in Redacted by agreement, which also houses Redacted by agreement and several other investigators working in the area of substance abuse. In particular, Dr. Platt is part of a core group of researchers who oversee NIDA-

and NIAAA-funded NHP research labs, including Redacted by agreement

Redacted by agreement

FACILITIES AND OTHER RESOURCES: Redacted

Redacted by agreement

Other Available Resources:

Redacted by agreement

MAJOR EQUIPMENT – UMMC

Three rooms have been allocated exclusively for the NHP behavioral pharmacology studies in this grant. All studies will be conducted in the monkeys' home cages. The cages have been modified to accept custom-designed operant drinking panels or custom-designed alcohol discrimination boxes. The component operant equipment consists of levers (retractable and non-retractable), LED lights, food pellet dispensers, retractable sippers equipped with solenoids, and connection panels (Med Associates, Inc.). The drinking panels also are equipped with shelving to hold fluid reservoirs. For these studies, 30 cages with drinking panels/discrimination boxes will be available.

Three rooms have been allocated exclusively for the rodent behavioral pharmacology studies in this grant. Two of the rooms contain standard operant conditioning chambers (Med Associates, Inc.; N=24 available chambers) equipped with response levers, stimulus lights, liquid troughs, and pumps for liquid delivery. For alcohol deprivation effect studies (conducted in the home cages) a minimum of 150 volumetric drinking tubes (Med Associates, Inc.) are available for use at any given time.

Precision balances are available in the shared wet laboratory space for preparing drug solutions. An Analox AM1 Series analyzer also is available in this space for determination of BALs.

MAJOR EQUIPMENT – Redacted

Redacted by agreement

Redacted by agreement

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Donna Middle Name M Last Name*: Platt Suffix:

Position/Title*: Professor
Organization Name*: UNIVERSITY OF MISSISSIPPI MED CTR
Department:
Division:
Street1*: 2500 North State Street
Street2:
City*: Jackson
County:
State*: MS: Mississippi
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 39216-4505

Phone Number*: 601 984 5896 Fax Number:

E-Mail*: dplatt@umc.edu

Credential, e.g., agency login: eRA Commo

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Platt_biosketch_-_Feb_2020_-_edited.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

PROFILE - Senior/Key Person

Redacted by agreement

Personal Info

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

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: Donna M. Platt

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

POSITION TITLE: 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
Franklin & Marshall College, Lancaster, PA	B.A.	1991	Biology
University of Massachusetts, Amherst, MA	M.A.	1994	Zoology
University of Massachusetts, Amherst, MA	Ph.D.	1997	Zoology
Harvard Medical School/NEPRC, Southborough, MA	Postdoctoral	2002	Behavioral Biology

A. Personal statement. Goals of the proposed research are to investigate specific GABAergic mechanisms underlying the abuse-related effects of alcohol, identify potential receptor targets for the development of alcohol phamacotherapies, and determine clinical scenarios in which specific pharmacological approaches are predicted to produce improved patient outcomes. Specifically, we plan to evaluate the contribution of γ- and δ-containing α4GABA$_A$ and α6GABA$_A$ receptor mechanisms to the subjective and reinforcing effects of alcohol in rhesus monkeys, and to the relapse-inducing effects of alcohol in rats. We then plan to evaluate selective GABAergic ligands as pharmacological adjuncts to behavioral therapy using a novel resurgence model of contingency management therapy. The current application builds logically on prior work conducted in my laboratory, and continues to involve my long-term co-investigator (Dr. Rowlett) and collaborator (Dr. Cook), who provide additional expertise in behavioral pharmacology, GABA pharmacology and GABA drug discovery and development. Personally, I have a broad background in basic primate behavior, as well as behavioral pharmacology, with specific training and expertise in key research areas for this application. As a graduate student at the University of Massachusetts-Amherst, I was trained as a primatologist and learned the behavioral observation techniques that serve as the foundation of the observation studies we propose in Aims 1 and 2 of this application. As a postdoctoral fellow at the New England Primate Research Center (NEPRC), I gained an understanding of the use of nonhuman primate models to study the behavioral consequences of drug action, becoming familiar with many operant procedures including drug discrimination, self-administration and reinstatement. As an Instructor at NEPRC, my research focus expanded beyond the behavioral effects of abused drugs to encompass the abuse-related effects of alcohol. Since moving my laboratory to the University of Mississippi Medical Center in 2013, I have established a rodent behavioral pharmacology lab that utilizes several models of alcohol self-administration and relapse. I also have become more familiar with the behavior analysis field and it is through this interaction that our novel model of contingency management was conceived. In summary, I have a demonstrated record of accomplished and productive research projects in an area of high relevance for the treatment of alcohol use disorders, and my expertise and experience have prepared me to lead the proposed project.

B. Positions and Honors

1. Professional Positions

1991-1995 Teaching Assistant, Department of Zoology, University of Massachusetts, Amherst, MA

1992-1996 Research Assistant, Department of Psychology, University of Massachusetts, Amherst, MA

1995-1997	Research Consultant, Laboratory of Behavioral Pharmacology, NERPRC, Southborough, MA
1996-1997	Research Assistant, Division of Behavioral Biology, NERPRC, Southborough, MA
1997-2002	Research Fellow in Psychobiology, Department of Psychiatry, Harvard Medical School
2002-2009	Instructor in Psychobiology, Department of Psychiatry, Harvard Medical School
2009-2013	Assistant Professor of Psychiatry, Department of Psychiatry, Harvard Medical School
2013-2018	Associate Professor, Department of Psychiatry & Human Behavior, University of Mississippi Medical Center
2014-2018	Associate Professor, Department of Neurobiology & Anatomical Sciences, University of Mississippi Medical Center
2014-2016	Associate Director, Graduate Program in Neuroscience, University of Mississippi Medical Center
2016-2019	Director, Graduate Program in Neuroscience, University of Mississippi Medical Center
2018-	Professor with Tenure, Department of Psychiatry & Human Behavior, University of Mississippi Medical Center
2018-	Director, Division of Neurobiology & Behavior Research, Department of Psychiatry & Human Behavior, University of Mississippi Medical Center

2. *Honors, Committees*

1987-1991	John Marshall Scholar
1991	Black Pyramid Senior Honor Society
1998	Recipient of College on Problems of Drug Dependence Travel Fellowship
1999	Recipient of National Institute of Mental Health Training Grant Stipend "Research Training –Biological Sciences
2001	Recipient of FASEB Summer Research Conference Travel Award to attend "Commonalities and Differences in the Mechanisms of Alcohol and Other Drugs of Abuse" conference in Tucson, AZ, and give presentation "Role of $GABA_A/\alpha 1$ Receptor Mechanisms in the Discriminative Stimulus Effects of Ethanol in Squirrel Monkeys".
2010	Center for Scientific Review, RC4 Challenge Grants - Recovery Act Limited Competition: Director's Opportunity for Research in Five Thematic Areas, Stage 1 reviewer
2010	National Institute on Alcohol Abuse and Alcoholism, Special Emphasis Panel: Review/Reverse Site Visit of ARC Grant Application (P60), ZAA1 GG(92), Ad Hoc member
2012	Center for Scientific Review, Neurotoxicology and Alcohol Study Section, NAL, Ad Hoc member
2013	The French National Research Agency (ANR), Programme de Recherche Translationnelle en Santé
2014	National Institute on Alcohol Abuse and Alcoholism, Special Emphasis Panel: Neuroscience Review Subcommittee, AA4, Ad Hoc member
2014	UMMC Excellence in Research Award – Silver
2009 - present	Center for Scientific Review, Neurobiology of Motivated Behavior Study Section, NMB, Ad Hoc member (Summer 2009; Spring 2013); Charter member (**Summer 2014 - present**);
2015	National Institute on Alcohol Abuse and Alcoholism, Special Emphasis Panel: Review/Reverse Site Visit of ARC Grant Applications (P50/P60), ZAA1 GG(69), ZAA1 GG(71), Ad Hoc member
2016	National Institute on Alcohol Abuse and Alcoholism, Special Emphasis Panel – Target of Low Dose Alcohol, ZAA1 JJ(08), Ad Hoc member
2016	UMMC Excellence in Research Award – Gold
2017	National Institute on Alcohol Abuse and Alcoholism, Special Emphasis Panel: Review/Reverse Site Visit of ARC Grant Applications (P50/P60), ZAA1 GG(69), Ad Hoc member

C. Contributions to Science

1. ***Ethanol—Pharmacology and Genetics/Genomics—Non-human Primates:*** My long-standing interest in understanding the addiction-related effects of ethanol has focused in two areas: (1) Understanding the role of GABAergic and opioid receptor systems and (2) evaluating the role of neurogenetic variation in the responsiveness to both ethanol as well as ethanol pharmacotherapies. In our pharmacology program, we have identified a potential novel target for developing a new and highly effective drinking cessation therapy (α5GABA$_A$ receptors). With respect to neurogenetics, we have found that the mu opioid receptor single-nucleotide polymorphism (C77G in nonhuman primates) plays a role in naltrexone responsiveness to attenuation of ethanol drinking and, importantly, is a key risk factor in determining sensitivity to ethanol's reinforcing effects.

   a) **Platt DM**, Duggan A, Spealman RD, Cook JM, Li X, Yin W and Rowlett JK. Contribution of α1GABA$_A$ and α5GABA$_A$ receptor subtypes to the discriminative stimulus effects of ethanol in squirrel monkeys. J Pharmacol Exp Ther 2005; 313: 658-67. (PMC unavailable).
   b) Rüedi-Bettschen D, Rowlett JK, Rallapalli S, Clayton T, Cook JM, **Platt DM**. Modulation of α5 subunit-containing GABA$_A$ receptors alters alcohol drinking by rhesus monkeys. Alcohol Clin Exp Res 2013; 37:624-34. (PMC3951841).
   c) Sawyer E, Moran C, Sirbu M, Szafir M, Van Linn M, Namjoshi O, Tiruveedhula VVPB, Cook JM, **Platt DM**. Little evidence of a role for α1GABA$_A$ subunit-containing receptor in a rhesus monkey model of alcohol drinking. Alcohol Clin Exp Res 2014; 38:1108-1117. (PMC3984357).
   d) Chandler CM, Overton JS, Rüedi-Bettschen D, **Platt DM** (2018) GABA$_A$ receptor subtype mechanisms and the abuse-related effects of ethanol: Genetic and pharmacological evidence. In: Grant KA, Lovinger DM, eds. *Handbook of Experimental Pharmacology: The Neural Circuitry of Alcohol*, pp. 3-27, Springer, Berlin, Heidelberg.
   e) Berro LF, Rüedi-Bettschen D, Cook JE, Golani LK, Li G, Jahan R, Rashid F, Cook JM, Rowlett JK, **Platt DM**. GABA$_A$ receptor subtypes and the abuse-related effects of ethanol in rhesus monkeys: Experiments with selective positive allosteric modulators. Alcohol Clin Exp Ther 2019; 43:791-802 (PMC6601614).

2. ***Primate Behavior, Cognition and the Study of Drugs:*** My area of study in graduate school was zoology, with a particular focus on species-typical behavior and ethologically-relevant cognitive processes in non-human primates. Part of my thesis work was conducted at the NIH Animal Center in Poolesville, MD, where I assessed novelty-seeking behavior in large groups of corral-housed rhesus monkeys. We continue to use novelty perception tasks and other cognitive tasks in our current research. Other parts of my thesis work were conducted at the New England Primate Research Center (NEPRC), where I met and eventually joined the laboratories of Dr. Jack Bergman and Dr. Roger Spealman. Early on, I sought to incorporate my expertise in behavioral observation and cognition techniques with the study of psychoactive drugs. We have developed and validated over the years quantitative behavioral observation techniques for squirrel monkeys, rhesus monkeys, vervet monkeys and pig-tail macaques. This work has led to significant contributions to our understanding of the mechanisms of action underlying the behavioral effects of stimulants, benzodiazepine-like drugs, and alcohol.

   a) **Platt DM**, Novak MA. Perception of novel changes in a familiar environment by socially-housed rhesus monkeys. Am J Primatol 1999; 47:117-31. (PMC unavailable).
   b) **Platt DM**, Rowlett JK, Spealman RD. Dissociation of cocaine-antagonist properties and motoric effects of the D1 receptor partial agonists SKF 83959 and SKF 77434. J Pharmacol Exp Ther 2000; 293:1017-26. (PMC unavailable).
   c) Rowlett JK, **Platt DM**, Lelas S, Atack JR, Dawson GR. Different GABA$_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci USA 2005; 102:915-20. (PMC545524).
   d) Chandler CM, Follett ME, Porter NJ, Liang KY, Vallender EJ, Miller GM, Rowlett JK, **Platt DM**. Persistent negative effects of alcohol drinking on aspects of novelty-directed behavior in male rhesus macaques. Alcohol 2017; 63:19-26 (PMC5584881).
   e) Duke AN, Meng Z, **Platt DM**, Atack JR, Dawson GR, Reynolds DS, Phani Babu Tiruveedhula VVN, Li G, Stephen MR, Sieghart W, Cook JM Rowlett JK. Evidence that sedative effects of benzodiazepines

involve unexpected $GABA_A$ receptor subtypes: Quantitative observation studies in rhesus monkeys. J Pharmacol Exp Ther 2018; 366:145-157 (PMC5988000).

3. ***Behavioral Pharmacology – Rodent Models:*** Since the move of my laboratory to UMMC, I have been able to establish a rodent laboratory focused on operant and non-operant models of the abuse-related effects of alcohol and other drugs. Rodent studies complement my nonhuman primate research program by bringing additional models and additional flexibility in pursuing more cellular/molecular questions. We believe that the observation of common outcomes across species and procedures raises the likelihood of similar results in humans. In addition, it has opened up collaborative opportunities with other investigators pursuing research in rodents.

   a) Gunter BW, Jones SA, Paul IA, **Platt DM**, Rowlett JK. Benzodiazepine and neuroactive steroid combinations in rats: Anxiolytic-like and discriminative stimulus effects. Psychopharmacology 2016; 233: 3237-3247. (PMC6334648).
   b) Rüedi-Bettschen D, **Platt DM**. Detrimental effects of self-administered methamphetamine during pregnancy on offspring development in the rat. Drug Alcohol Depend 2017; 177:171-177. (PMC5701573).
   c) Chandler CM, Reeves-Darby J, Jones SA, McDonald JA, Li G, Rahman MT, Cook JM, **Platt DM**. α5$GABA_A$ subunit-containing receptors and sweetened alcohol cue-induced reinstatement and active sweetened alcohol self-administration in male rats. Psychopharmacology 2019; 236:1797-1806. (PMC6606346).
   d) Cook JE, Chandler CM, Rüedi-Bettschen D, Taylor I, Patterson SC, **Platt DM**. Changes in the elimination and resurgence of alcohol-maintained behavior in rats and the effects of naltrexone. Psychol Addict Behav 2020; 34:10-22. (PMCID: 7007344).
   e) Chandler CM, Reeves-Darby J, Jones SA, Li G, Rahman MT, Cook JM, **Platt DM**. Modulation of relapse-like drinking in rats by ligands targeting the α5$GABA_A$ receptor. Addict Biol Depend under review.

4. ***Abuse of stimulants and opioids***: My work on the abuse-related effects of stimulants, primarily cocaine, has covered topics such as medications development for abuse, neuropharmacological mechanisms underlying reinstatement of cocaine seeking, and cocaine-opioid ("speedball") addiction. Our work on medications development has focused on dopaminergic, serotonergic, noradrenergic and glutamatergic targets of both cocaine taking and reinstatement, the latter serving as a model of relapse. Our research with speedballs has shown that mu opioid drugs often share effects with cocaine, primarily via a dopaminergic action.

   a) **Platt DM**, Rowlett JK, Spealman RD. Behavioral effects of cocaine and dopaminergic strategies for preclinical medication development. Psychopharmacology 2002; 163:265-282. (PMC unavailable).
   b) **Platt DM**, Rowlett JK, Spealman RD. Noradrenergic mechanisms in cocaine-induced reinstatement of drug seeking in squirrel monkeys. J Pharmacol Exp Ther 2007; 322: 894-902. (PMC unavailable).
   c) Rowlett JK, **Platt DM**, Yao W-D, Spealman RD. Modulation of heroin and cocaine self-administration by dopamine D1- and D2-like receptor agonists in rhesus monkeys. J Pharmacol Exp Ther 2007; 321: 1135-1143. (PMC unavailable).
   d) Rüedi-Bettschen D, Spealman RD, **Platt DM**. Attenuation of cocaine-induced reinstatement of drug seeking in squirrel monkeys by direct and indirect activation of 5-HT2C receptors. Psychopharmacology 2015; 232:2959-2968. (PMC4515185).
   e) Unpublished

Complete list of published works in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/donna.platt.1/bibliography/public/

D. Research Support

1. Ongoing Research Support

R01 DA011792 Rowlett (PI) 3/2015-12/2019 (NCE – renewal percentile 6%)
NIH/NIDA
Anxiolytic and Abuse-Related Effects of BZ Ligands
The long-term objective of this project is to elucidate receptor mechanisms that underlie the anxiolytic versus the abuse-related effects of benzodiazepine (BZ) ligands. Our strategy is to use BZ ligands that vary in receptor selectivity and/or agonist efficacy as probes for assessing mechanisms underlying the anti-conflict effects and self-administration in rhesus monkeys. Role: Co-Investigator

R01 DA039167 Freeman (PI) 9/1/15-8/31/20
NIH/NIDA
Deterrents for prescription opioid abuse
The goal of this project is to investigate the utility of kappa opioid receptor agonists as deterrents to the abuse of oxycodone and other prescription opioids using monkey and rat models of reinforcing effects. To ensure that kappa agonists do not alter the therapeutic effects of mu agonists, antinociception is also studied. Role: Co-Investigator

R01 DA043204 Rowlett (PI) 7/1/17-6/30/22
NIH/NIDA
Tolerance and physical dependence after chronic benzodiazepine treatment
The goal of this project is to investigate the GABA-A receptor subtype mechanisms contributing to the development of tolerance and dependence after chronic exposure to benzodiazepines. Role: Co-Investigator

2. Completed Research Support in last 5 years

R01 AA016179 Platt (PI) 6/2006-8/2017 (NCE)
NIH/NIAAA
GABA-A Receptor Subtype Mechanisms in Nonhuman Primate Models of Alcohol Abuse
The purpose of this proposal is to investigate the role of GABA-A receptor mechanisms in nonhuman primate models of the interoceptive, reinforcing, and relapse-inducing effects of alcohol. Role: PI

1 R01 AG035361 Rowlett (PI) 7/2010-6/2015
NIH/NIA Novel GABA-A Modulators as Cognitive Enhancers
The overall goal of this application is to explore the potential for new compounds, acting at a brain protein called the "GABA receptor", to enhance cognitive function. Role: Co-Investigator

1 R01 DA033795 Rowlett (PI) 7/2012-6/2017 (NCE)
NIH/NIDA Neurosteroid-BZ combinations: Strategy for reducing abuse and sedation
The overall goal of this application is to explore the potential for a novel combination strategy to enhance the therapeutic efficacy of benzodiazepines without concomitant increases in side effects. Role: Co-Investigator

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Redacted by agreement

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 9288244730000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Donna	M	Platt		PD/PI		EFFORT			44,250.00	12,213.00	56,463.00
2.		Redacted by agreement				Co-Investigator					9,865.00	2,723.00	12,588.00
3.						Co-Investigator					13,260.00	3,660.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,971.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			27,378.00	7,556.00	34,934.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Researcher II				13,865.00	3,827.00	17,692.00
1	Researcher II				33,500.00	9,246.00	42,746.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**95,372.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**181,343.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel		**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)		4,000.00
2. Foreign Travel Costs		
	Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	
3. Consultant Services	4,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	49,452.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Care Costs	41,639.00
9 . Monkey Purchases	45,000.00
10 . Rat purchases	4,540.00
Total Other Direct Costs	**160,131.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**345,474.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.0	321,022.00	176,562.00
		Total Indirect Costs	**176,562.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**522,036.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	522,036.00

L. Budget Justification*	File Name: Platt_budget_justification_-_Feb_2020_-_edited.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 9288244730000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Donna	M	Platt		PD/PI		EFFORT			44,250.00	12,213.00	56,463.00
2.		Redacted by agreement				Co-Investigator					9,865.00	2,723.00	12,588.00
3.						Co-Investigator					13,260.00	3,660.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,971.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			28,440.00	7,849.00	36,289.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Researcher II				13,865.00	3,827.00	17,692.00
1	Researcher II				33,500.00	9,246.00	42,746.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**96,727.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**182,698.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	
3. Consultant Services	4,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	49,498.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Care Costs	54,210.00
9 . Monkey Purchases	22,500.00
10 . Rat Purchases	4,540.00
Total Other Direct Costs	**150,248.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**336,946.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.0	287,448.00	158,096.00
		Total Indirect Costs	**158,096.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**495,042.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	495,042.00

L. Budget Justification* File Name: Platt_budget_justification_-_Feb_2020_-_edited.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 9288244730000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Donna	M	Platt		PD/PI		EFFORT			44,250.00	12,213.00	56,463.00
2.		Redacted by agreement				Co-Investigator					9,865.00	2,723.00	12,588.00
3.						Co-Investigator					13,260.00	3,660.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,971.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			29,550.00	8,156.00	37,706.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Researcher II				13,865.00	3,827.00	17,692.00
1	Researcher II				33,500.00	9,246.00	42,746.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**98,144.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**184,115.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	
3. Consultant Services	4,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	49,545.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Care Costs	79,351.00
9 . Monkey Purchases	45,000.00
10 . Rat Purchases	4,540.00
Total Other Direct Costs	**197,936.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**386,051.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.0	336,506.00	185,078.00
		Total Indirect Costs	**185,078.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**571,129.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	571,129.00

L. Budget Justification* File Name: Platt_budget_justification_-_Feb_2020_-_edited.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 9288244730000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Donna	M	Platt		PD/PI		EFFORT			44,250.00	12,213.00	56,463.00
2.		Redacted by agreement				Co-Investigator					9,865.00	2,723.00	12,588.00
3.						Co-Investigator					13,260.00	3,660.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,971.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			30,654.00	8,461.00	39,115.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Researcher II				13,865.00	3,827.00	17,692.00
1	Researcher II				33,500.00	9,246.00	42,746.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**99,553.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**185,524.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	
3. Consultant Services	4,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	49,592.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Care Costs	97,499.00
9 . Rat Purchases	4,767.00
Total Other Direct Costs	**171,358.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**360,882.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.0	311,290.00	171,210.00
		Total Indirect Costs	**171,210.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**532,092.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	532,092.00

L. Budget Justification*: File Name: Platt_budget_justification_-_Feb_2020_-_edited.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 9288244730000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Donna	M	Platt		PD/PI		EFFORT			44,250.00	12,213.00	56,463.00
2.		Redacted by agreement				Co-Investigator					9,865.00	2,723.00	12,588.00
3.						Co-Investigator					13,260.00	3,660.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,971.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			30,654.00	8,461.00	39,115.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Researcher II				13,865.00	3,827.00	17,692.00
1	Researcher II				33,500.00	9,246.00	42,746.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**99,553.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**185,524.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 9288244730000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF MISSISSIPPI MED CTR

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	
3. Consultant Services	4,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	49,638.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Care Costs	97,499.00
Total Other Direct Costs	**166,637.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**356,161.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.0	306,523.00	168,588.00
		Total Indirect Costs	**168,588.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**524,749.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	524,749.00

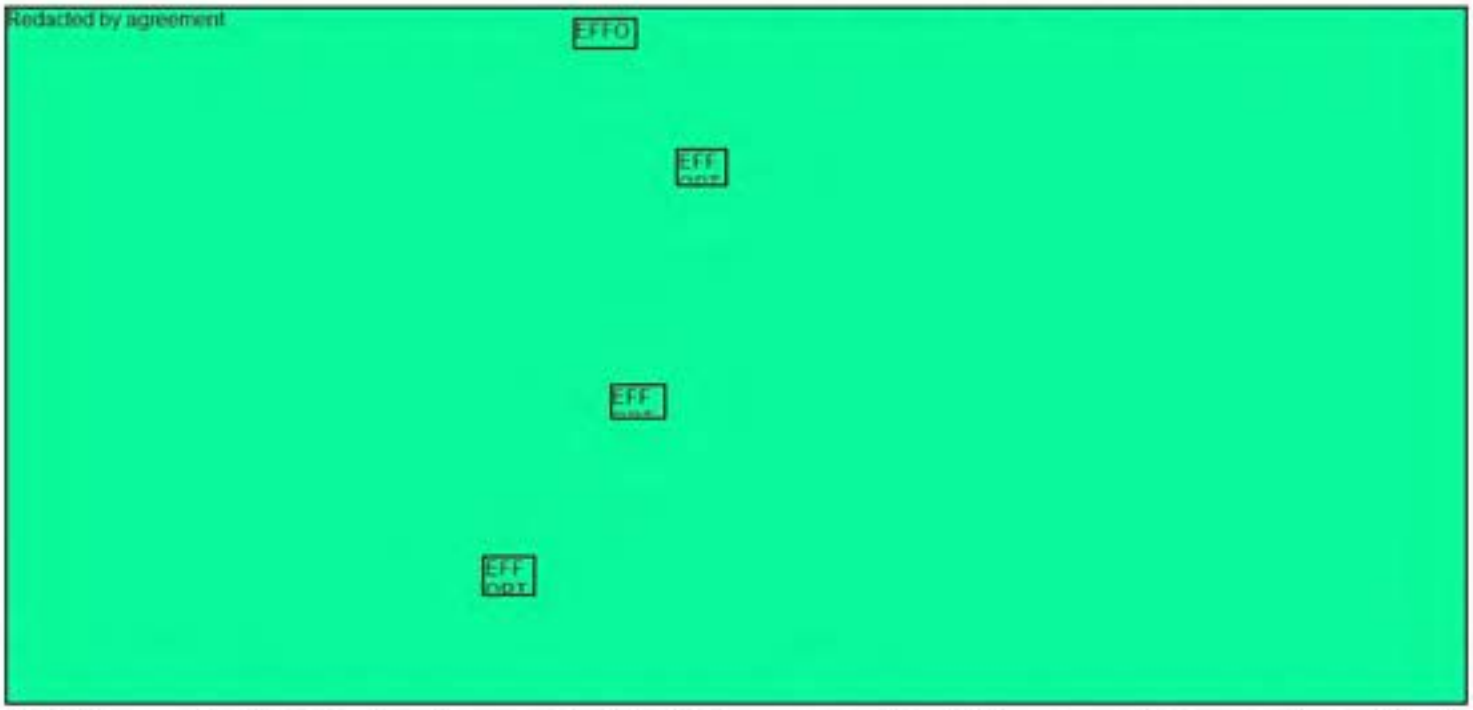
L. Budget Justification* File Name: Platt_budget_justification_-_Feb_2020_-_edited.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

BUDGET JUSTIFICATION

1. Personnel:

Donna M. Platt, Ph.D., Principal Investigator (EFF calendar months). Dr. Platt is Professor in the Department of Psychiatry & Human Behavior at UMMC. As Principal Investigator, Dr. Platt will be responsible for the overall administration and direction of this project. This will involve coordination with Drs. Rowlett and Rüedi-Bettschen for initiation and maintenance of the studies, supervision of data collection, and preparation of manuscripts. Dr. Platt is the co-mentor for the Postdoctoral Fellow on this project (Dr. JE Cook).



TBN, Researcher II (12.0 calendar months). We will hire a research technician to provide day-to-day technical support for the cue-induced reinstatement and alcohol deprivation effect studies.

2. Travel:

Travel expenses ($4,000/year) are requested to defray costs for one or two of the investigative team to attend a domestic scientific conference (e.g., Research Society on Alcoholism, Society for Neuroscience) each year to present the latest results from this project.

3. Other direct costs:

Materials and supplies:

Supply purchases for this project ($15,000/year) consist of general laboratory supplies typical for establishing and maintaining behavioral studies in rhesus monkeys and rats.

Itemized laboratory supplies for Project Year 1.				
Item	**Vendor**	**Cost/Unit**	**Units/Year**	**Total for Year 1**
PPE, syringes, saline, and gauze	Internal			$3500
Gaboxadol	Tocris	$145	45	$6525
Ethanol	Pharmaco	$75	30	$2250
Precisions Pellets	Bioserve	$75	10	$750
Single lumen swivels	Lomir	$107	5	$535
Monkey jackets	Lomir	$107	7	$749
Jacket tethers	Lomir	$79	5	$395
Total=				**$14,704**

Animal care costs:
A significant portion of each year's budget consists of per diems for monkeys and rats.

Per diem rates for monkeys are $5.74/monkey/day. The number of monkeys carried on this grant will increase across the years as monkeys are purchased and studies initiated.

Year 1: 12 monkeys - $25,141
Year 2: 18 monkeys - $37,712
Years 3-5: 30 monkeys - $62,853

Per diem rates for rats are $1.13/cage/day. In years 1-3, rats will be pair-housed for reinstatement studies; in years 4-5, rats will be individually-housed for alcohol deprivation effect studies.
Year 1-3: 40 cages - $16,498
Year 4-5: 84 cages - $34,646

Total animal care costs (monkey + rat):
Year 1: $41,639
Year 2: $54,210
Year 3: $79,351
Year 4: $97,499
Year 5: $97,499

Consultant service:
Consultant expenses ($4,500/year) are requested for Dr. James Cook, University Distinguished Professor, University of Wisconsin-Milwaukee and sub-award principal investigator.

Animal purchases:
The standard cost of experimentally naïve, healthy rhesus monkeys is $7,500. Monkeys will be purchased in Years 1-3 of the project (see below).

Year 1: 6 monkeys (females; alcohol self-administration) - $45,000
Year 2: 3 monkeys (females; alcohol discrimination) - $22,500
Year 3: 6 monkeys (females; sucrose self-administration) - $45,000

The cost of experimentally naïve Wistar rats is $56.10-$57.40 (depending on sex). Reinstatement studies will be conducted in years 1-3 (train rats and test two experimental compounds/year); alcohol deprivation effect studies will be conducted in years 4-5 (all drugs can be tested in separate groups at the same time).

Year 1: 80 rats (males and females; alcohol and sucrose reinstatement) - $4540
Year 2: 80 rats (males and females; alcohol and sucrose reinstatement) - $4540
Year 3: 80 rats (males and females; alcohol and sucrose reinstatement) - $4540
Year 4: 84 rats (males and females; alcohol deprivation effect) - $4767

4. Subcontract:
This application includes a subcontract to the Redacted by agreement for $50,000/year (direct costs + indirect costs). This subcontract covers synthesis (materials and technical personnel) of the majority of compounds to be used in this application (see Scope of Work).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		429,855.00
Section B, Other Personnel		489,349.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		919,204.00
Section C, Equipment		0.00
Section D, Travel		20,000.00
1. Domestic	20,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		846,310.00
1. Materials and Supplies	75,000.00	
2. Publication Costs	0.00	
3. Consultant Services	22,500.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	247,725.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	370,198.00	
9. Other 2	117,267.00	
10. Other 3	13,620.00	
Section G, Direct Costs (A thru F)		1,785,514.00
Section H, Indirect Costs		859,534.00
Section I, Total Direct and Indirect Costs (G + H)		2,645,048.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		2,645,048.00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 6279063990000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Redacted by agreement

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				PD/PI		EFFORT			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	EFFORT			15,327.00	3,081.00	18,408.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,408.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,408.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Instrument maintenance	1,600.00
9 . Student tuition	8,400.00
Total Other Direct Costs	**17,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**35,408.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.0	27,008.00	14,044.00
		Total Indirect Costs	**14,044.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Matthew Dito, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**49,452.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	49,452.00

L. Budget Justification*: File Name: Cook_budget_justification_-_Feb_2020.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 6279063990000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Redacted by agreement

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1		Redacted by agreement				PD/PI		EFFO			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	EFFORT			15,327.00	3,111.00	18,438.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,438.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,438.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost **0.00**

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs** **0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Instrument maintenance	1,600.00
9 . Student tuition	8,400.00
Total Other Direct Costs	**17,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**35,438.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.0	27,038.00	14,060.00
		Total Indirect Costs	**14,060.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Matthew Dito, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**49,498.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	49,498.00

L. Budget Justification* File Name: Cook_budget_justification_-_Feb_2020.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 6279063990000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Redacted by agreemnnt

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1		Redacted by agreement				PD/PI		EFFO RT			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	EFFORT			15,327.00	3,142.00	18,469.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,469.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,469.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Instrument maintenance	1,600.00
9 . Student tuition	8,400.00
Total Other Direct Costs	**17,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**35,469.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.0	27,069.00	14,076.00
		Total Indirect Costs	**14,076.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Matthew Dito, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**49,545.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	49,545.00

L. Budget Justification* File Name: Cook_budget_justification_-_Feb_2020.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Tracking Number: GRANT13085906 Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 6279063990000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Redacted by agreement

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				PD/PI		EFFORT			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	EFFORT			15,327.00	3,173.00	18,500.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,500.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,500.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 627906399000

Budget Type*: ○ Project ● Subaward/Consortium

Organization: Redacted by agreement

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost **0.00**

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs** **0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Instrument maintenance	1,600.00
9 . Student tuition	8,400.00
Total Other Direct Costs	**17,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**35,500.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.0	27,100.00	14,092.00
		Total Indirect Costs	**14,092.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Matthew Dito, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**49,592.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	49,592.00

L. Budget Justification* File Name: Cook_budget_justification_-_Feb_2020.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 6279063990000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization Redacted by agreement

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1		Redacted by agreement				PD/PI		EFFORT			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	EFFORT			15,327.00	3,203.00	18,530.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,530.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,530.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget (C-E) (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 6279063990000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Redacted by agreement

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Instrument maintenance	1,600.00
9 . Student tuition	8,400.00
Total Other Direct Costs	**17,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**35,530.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.0	27,130.00	14,108.00
		Total Indirect Costs	**14,108.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Matthew Dito, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**49,638.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	49,638.00

L. Budget Justification* File Name: Cook_budget_justification_-_Feb_2020.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Sub-award Budget Justification:

Personnel:

Redacted by agreement

Other Direct Costs:

Materials and Supplies:

Chemicals, solvents, silica gel, standard spectroscopy: $7,000/year

Maintenance:

Mass spectrometer: $1,600/year

Indirect Costs:

The Redacted by F&A rate of 52% is applied ($ 14,044).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		92,345.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		92,345.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		85,000.00
1. Materials and Supplies	35,000.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	8,000.00	
9. Other 2	42,000.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		177,345.00
Section H, Indirect Costs		70,380.00
Section I, Total Direct and Indirect Costs (G + H)		247,725.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		247,725.00

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	331,430	322,886	371,975	346,790	342,053	**1,715,134**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*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) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, 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) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Funding Opportunity Number: PA-19-056 . Received Date: 2020-04-28T12:18:24.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Platt_specific_aims_-_Feb_2020_-_edited.pdf
3. Research Strategy*	Platt_strategy_-_Feb_2020_-_edited.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Platt_vertebrate_animals_-_Feb_2020.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Platt_fully_executed_LOI_-_Feb_2020_Final.pdf
9. Letters of Support	Cook_letter_of_support_-_Feb_2020_-_edited.pdf
10. Resource Sharing Plan(s)	Platt_resource_sharing_plan_-_Feb_2020.pdf
11. Authentication of Key Biological and/or Chemical Resources	Platt_authentication_-_Feb_2020.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT13085906
Funding Opportunity Number: PA-19-056. Received Date: 2020-04-28T12:18:24.000-04:00

1. SPECIFIC AIMS

The development of pharmacological and behavioral strategies that universally reduce alcohol consumption and/or eliminate craving continues to be a challenge for researchers. Alcohol's ability to potentiate the activity of γ-aminobutyric acid (GABA) at $GABA_A$ receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both human subjects and laboratory animals, making this system an attractive candidate for the development of therapeutics. **The complex molecular biology of $GABA_A$ receptors raises the possibility that subtype-selective agents might be developed with therapeutic specificity against alcohol use disorders (AUDs). It is this basic premise that has guided the studies conducted in our laboratory over the past 15 years.**

Our previous research has focused on $GABA_A$ receptor subtypes that are sensitive to classic benzodiazepine-type ligands (i.e., receptors containing α1, α2, α3, or α5 subunits; α1$GABA_A$ receptors, α2/3$GABA_A$ receptors, and α5$GABA_A$ receptors, respectively), and we have shown that α5$GABA_A$ and α2/3$GABA_A$, but not α1$GABA_A$, receptors play a key role in the reinforcing and discriminative stimulus (DS) effects of alcohol in monkeys and the relapse-like effects of alcohol in rats. However, alcohol has been shown to modulate not only these "diazepam-sensitive" receptors, but also those insensitive to benzodiazepines (i.e., $GABA_A$ receptors containing α4 and α6 subunits). $GABA_A$ receptors containing α4 or α6 subunits are comprised of β subunits, and either a γ or δ subunit. Important differences exist between these populations of "diazepam-insensitive" receptors. In this regard, γ-containing receptors (i.e., α4γ$GABA_A$ and α6γ$GABA_A$ receptors) are located synaptically and extrasynaptically and mediate both phasic and tonic inhibition of neurons, whereas δ-containing receptors (i.e., α4δ$GABA_A$ and α6δ$GABA_A$ receptors) are located extrasynaptically and contribute to tonic inhibition only. Our prior findings indicate a clear role for several extrasynaptically and synaptically located $GABA_A$ receptors in alcohol's behavioral effects. However, the contribution of extrasynaptically located α4βδ and α6βδ $GABA_A$ receptors remains controversial, with the literature supporting both a facilitative and inhibitory role for these receptors. Thus, **goals of the proposed research are to resolve the role of γ- vs. δ-containing α4 and α6$GABA_A$ receptors in the DS, reinforcing and relapse-inducing effects of alcohol and determine whether ligands targeting these receptors exhibit therapeutic specificity against alcohol-maintained behavior**.

In the clinic, pharmacotherapies typically are one of the last options to be offered to the AUD patient; rather, behavioral therapies are the approach of choice. However, patient outcomes generally are improved when behavioral therapies are combined with adjunctive pharmacotherapies. To model "medication-assisted treatment", we have developed a resurgence model of contingency management therapy and have obtained exciting preliminary data with the FDA-approved pharmacotherapy naltrexone. Thus, **a final goal of our proposed research is to apply what we have learned previously regarding the therapeutic utility of selective GABAergic drugs alone and begin to evaluate these drugs as adjunctive pharmacotherapies to augment behavioral therapy.**

Specific Aim 1 will establish the involvement of γ-containing vs. δ-containing α4 and α6$GABA_A$ receptors in the DS, reinforcing and relapse-inducing effects of alcohol. We will use novel ligands selective for γ- vs. δ-containing α4 and α6$GABA_A$ receptors and models of interoceptive effects, alcohol seeking and taking established in our laboratory with rodent and primate species. We hypothesize that δ-containing $GABA_A$ receptors are key mediators of the reinforcing, but not DS or relapse-inducing, effects of alcohol. A novel contribution of this project will be to delineate the roles of α4δ, α6δ, α4γ, and α6γ$GABA_A$ receptors, for which very little behavioral data exists.

Specific Aim 2 will establish the potential therapeutic utility of α4δ, α6δ, α4γ, and α6γ$GABA_A$ ligands by determining the extent to which they alter performance maintained by a nondrug reinforcer (sucrose) and/or induce undesirable side effects using a quantitative observation procedure.

Specific Aim 3 will investigate the utility of selective GABAergic ligands with favorable side effect profiles (identified in current and past project periods) to serve as adjunctive pharmacotherapies in a model of medication-assisted treatment. These studies will make use of a novel resurgence model of contingency management developed recently in our laboratory and, initially, evaluate ligands that either mimic (e.g., α2/3$GABA_A$ agonists) or attenuate (e.g., α5$GABA_A$ inverse agonists) the behavioral effects of alcohol.

Integration of results from the aims will continue to yield needed information about neuropharmacological mechanisms underlying the addictive effects of alcohol and begin to identify clinical scenarios in which specific pharmacological approaches might improve patient outcomes.

2. SIGNIFICANCE

2a. Background: Over the last 10-20 years, the prevalence of alcohol use, high-risk drinking and clinically-defined alcohol use disorder (AUD) has increased significantly in the U.S. population[1]. The misuse of alcohol is associated with debilitating social, psychological and physical health problems[2], which place an extreme financial burden on individuals, families, and society as a whole[3]. While both medication-based and behavioral therapies are available to treat alcohol-related disorders, no universally effective strategy has been identified.

Alcohol's ability to potentiate the activity of γ-aminobutyric acid (GABA) at $GABA_A$ receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both human subjects and laboratory animals[4-10], making this system an attractive candidate for the development of therapeutics. Molecular biological studies show that the mammalian $GABA_A$ receptor is a pentamer consisting of subunits from at least five different families, including the α, β, γ and δ subunits[11-14]. Many of these subunits have a number of different isoforms (e.g., α1-α6, β1-β3, γ1-γ3)[14]. **This molecular complexity raises the possibility that $GABA_A$ subtype-selective agents can be developed with therapeutic specificity for AUDs. It is this basic premise that has guided the studies in our laboratory for the last 15 years.**

2b. $GABA_A$ receptor subunits and the abuse-related effects of alcohol: The table provides a summary of the current state of knowledge regarding $GABA_A$ receptor subtypes and alcohol-induced behaviors. We (and others) have obtained considerable information on the role of several subtypes in specific abuse-related effects of alcohol. Below, we detail key results from our own experiments. In the table, question marks indicate where additional pharmacological research is needed. Note that the columns for α4- and α6-containing $GABA_A$ receptors contain all question marks, reflecting the lack of selective compounds to differentiate these subtypes (but see proposed studies in Aim 1). Likewise, the rows associated with relapse-inducing effects are filled mostly with question marks. To our knowledge, we are the only laboratory investigating the role of $GABA_A$ receptor subtypes in this defining aspect of AUDs.

Alcohol and $GABA_A$ Subtypes: Summary of our previous findings

	Subtype (αx subunit)				
Behavioral effect	**α1**	**α2/3**	**α4**	**α5**	**α6**
Discriminative Stimulus Effects	No	Yes	?	Yes	?
Self-Administration	No	Yes	?	Yes	?
Cue-Induced Reinstatement	?	?	?	Yes	?
Relapse-Like Drinking	?	?	?	Yes	?
Cue-Reactivity	?	?	?	Yes	?

"Yes" or "No" indicates involvement or no involvement of the indicated subtype.
"?" indicates incomplete or no data.

2bi. α1$GABA_A$ receptors: Rodent studies provide some evidence for a role for the α1$GABA_A$ receptor subtype in alcohol reinforcement. For example, in rats, self-administration of alcohol, but not saccharin or sucrose, is reduced by antagonists with selectivity for α1$GABA_A$ receptors[15-16]. In addition, genetically-altered mice lacking the α1$GABA_A$ receptor exhibit reduced preference for alcohol in a two-bottle choice procedure[17]. In monkeys, we found that α1$GABA_A$ agonists (i.e., zolpidem, zaleplon) reproduced, at least partially, the discriminative stimulus (DS) effects of alcohol[18-19], however, α1$GABA_A$ antagonists failed to alter alcohol's DS effects or the alcohol-like DS effects of zolpidem and zaleplon[19]. These results suggest that α1$GABA_A$ receptor mechanisms play little role in the DS effects of alcohol. Indeed, the finding of the α1$GABA_A$ agonists partially mimicking the DS effects of alcohol suggests that their capacity to stimulate *other* $GABA_A$ receptors might underlie this substitution pattern. In self-administration studies, none of the α1$GABA_A$ agonists or antagonists tested modulated alcohol self-administration up to doses that produced overt effects on observable behavior (e.g., ataxia) and/or disrupted operant behavior[20]. These findings suggest that α1$GABA_A$ receptor mechanisms do not play a predominant role in the reinforcing effects of alcohol in monkeys. For these reasons, we have not pursued additional studies focused on α1$GABA_A$ receptors and have shifted attention to other $GABA_A$ receptor subtypes.

2bii. α2/3$GABA_A$ receptors: Genetic association and transgenic mouse studies indicate a potential role for the α2$GABA_A$ and/or α3$GABA_A$ receptor subtypes in the incidence of AUDs, as well as in the subjective and reinforcing effects of alcohol[21-26], prompting us to hypothesize a facilitative role for α2/3$GABA_A$ receptors in the abuse-related effects of alcohol. In collaboration with Dr. James Cook, U Wisconsin-Milwaukee, we obtained strong support for this hypothesis in our monkey models of the subjective and reinforcing effects of alcohol with compounds possessing high intrinsic efficacy (defined by modulation of GABA-potentiated Cl- currents) at

α2GABA$_A$ and/or α3GABA$_A$ receptors, but lower intrinsic efficacy at α1GABA$_A$ and α5GABA$_A$ receptors (i.e., "functional selectivity" for α2/3GABA$_A$ receptors)[18]. We showed that the functionally-selective α2/3GABA$_A$ compounds HZ-166 and XHe-II-053 fully substituted for the DS effects of alcohol and enhanced oral alcohol self-administration[18]. Importantly, we found that YT-III-31 and YT-III-271, compounds with functional selectivity for only the α3GABA$_A$ receptor, also reproduced the alcohol DS and augmented alcohol self-administration. Our results with YT-III-31 and YT-III-271 allow us to conclude that α3GABA$_A$ receptor activation is *sufficient* for inducing alcohol-like subjective effects and facilitating its reinforcing effects[18]. Moreover, we found the functionally-selective α2/3GABA$_A$ and α3GABA$_A$ compounds to be without effect on sucrose self-administration[18]. This observation suggests a specific role for these subtypes in the reinforcing effects of alcohol. In observation studies, high doses of these compounds engendered increases in rest/sleep posture, a behavior associated with mild sedative effects[27], in both alcohol- and sucrose-drinking monkeys; a finding in agreement with literature suggesting a more general role for these subtypes in sedative-like behaviors (e.g., [28-30]).

2biii. α5GABA$_A$ receptors: Significant evidence from genetic association[31], transgenic mice[28], and pharmacological studies in rats[32-33] supports a potentially key role for α5GABA$_A$ receptor mechanisms in, at least, the reinforcing effects of alcohol. We have evaluated α5GABA$_A$ inverse agonists across diverse chemical series, in alcohol discrimination and self-administration procedures and have found that that these ligands both attenuate the DS effects of alcohol without significantly altering response rate[19] and reduce the reinforcing effects of alcohol, in some cases to an extent similar to the FDA-approved therapeutic naltrexone (Fig. 1, top; cf. [34-35]). Importantly, if a compound also decreased sucrose drinking, it only occurred at doses >3-fold higher than those which significantly reduced alcohol drinking, implying that there is a therapeutic window in which these compounds would be expected to selectively modulate the reinforcing effects of alcohol versus those of non-drug reinforcers.

To begin to address the critical issue of relapse associated with AUD, we established in rats a cue-induced reinstatement procedure to study environmental triggers of relapse, and the alcohol deprivation effect (ADE) model to study relapse-like drinking[36-37]. Both cue-induced alcohol seeking[36], and relapse-like drinking (Fig. 1, bottom), were attenuated by the α5GABA$_A$ inverse agonist L-655,708; conversely, the ADE and, in some cases, cue-induced alcohol-seeking behavior[36] were augmented by the α5GABA$_A$ agonist QH-ii-066. These lines of evidence, converging across procedures and species, support the notion that α5GABA$_A$ receptors may play a unique role in the abuse-related effects of alcohol and that α5GABA$_A$ receptor inverse agonists may have potential as anti-alcohol medications to both reduce active drinking and prevent relapse.

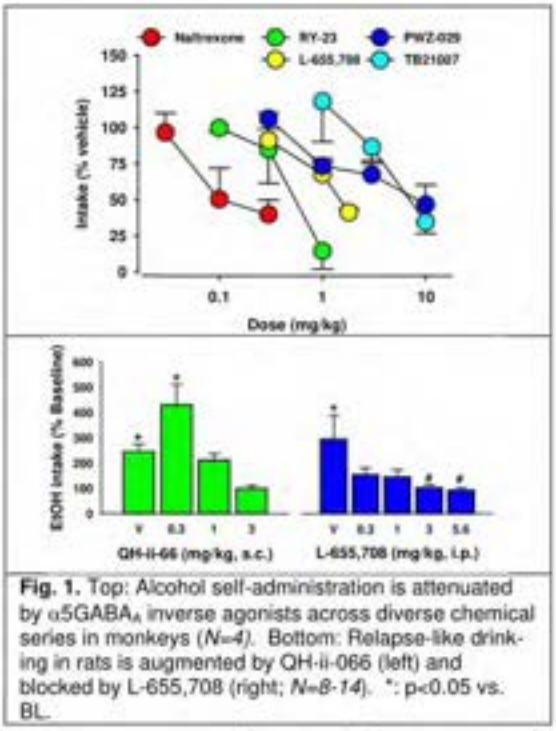


Fig. 1. Top: Alcohol self-administration is attenuated by α5GABA$_A$ inverse agonists across diverse chemical series in monkeys (*N=4*). Bottom: Relapse-like drinking in rats is augmented by QH-ii-066 (left) and blocked by L-655,708 (right; *N=8-14*). *: p<0.05 vs. BL.

2c. Scientific premise for proposed studies: As is evident from above, an overarching goal of our research program has been to provide relevant data to inform AUD medications discovery and development by targeting GABA$_A$ subtypes. We have been successful at isolating roles for specific receptor subtypes in mediating different abuse-related effects of alcohol. Moreover, we have identified compounds that target relevant subtypes and have reasonable side effect profiles. In Aims 1 and 2 of this proposal, we will extend our studies to α4- and α6-subunit containing GABA$_A$ receptors that may be uniquely involved in the effects of low-to-moderate doses of alcohol. In Aim 3, we will begin to apply the knowledge we have gained from our studies to evaluate the utility of GABAergic ligands as adjunctive therapies to more commonly used behavioral therapies (i.e., medication-assisted therapy or MAT).

2ci. Diazepam-insensitive GABA$_A$ receptor subtypes, alcohol, and current controversies: Classically, GABA$_A$ receptors have been characterized by their ability to be modulated by diazepam, with "diazepam-sensitive" receptors consisting of α1GABA$_A$, α2/3GABA$_A$, and α5GABA$_A$ receptors (for review see [14]). We and others have found key roles for several of these diazepam-sensitive receptors in multiple abuse-related effects of alcohol (for review see [4]). However, alcohol has been postulated to modulate not only diazepam-sensitive receptors,

but also those insensitive to classic benzodiazepines (i.e., $GABA_A$ receptors containing $\alpha4$ and $\alpha6$ subunits). $GABA_A$ receptors containing $\alpha4$ or $\alpha6$ subunits are comprised of β subunits, and either a γ or δ subunit. Important differences exist between these two populations of "diazepam-insensitive" receptors. γ-containing receptors (i.e., $\alpha4\gamma GABA_A$ and $\alpha6\gamma GABA_A$ receptors) are located synaptically and extrasynaptically and mediate both phasic and tonic inhibition of neurons; whereas δ-containing receptors (i.e., $\alpha4\delta GABA_A$ and $\alpha6\delta GABA_A$ receptors) are located extrasynaptically and contribute to tonic inhibition only[38-40]. To date, only $\alpha4\delta GABA_A$ and $\alpha6\delta GABA_A$ receptors have been studied in the context of alcohol pharmacology.

Although controversial, it has been suggested that δ-containing receptors are preferential mediators of behaviorally relevant, lower doses of alcohol[41-42] (but see [43-44]). Complementing this idea are findings showing that alcohol enhances tonic $GABA_A$ receptor-mediated current in several brain areas[40, 45-46], though this latter effect has not always been observed[47]. Questions persist regarding the role of δ-containing $GABA_A$ receptors in alcohol's behavioral effects, and as with the electrophysiological evidence, the behavioral evidence is inconclusive. In fact, the literature supports both a facilitative and inhibitory role for $\alpha4\delta$ and $\alpha6\delta GABA_A$ receptors.

Pharmacological approaches have been limited by a lack of selective drugs to probe these subtypes. To date, one of the only drugs that has been useful in this context is gaboxadol (also referred to as THIP), a partial agonist that acts preferentially at extrasynaptic $GABA_A$ receptors that co-express either the $\alpha4$ or $\alpha6$ subunit[48-49]. Gaboxadol has been shown to enhance acquisition of alcohol consumption[50] and increase intake and preference in rats under a 2-bottle choice procedure[51]. In monkeys, we have found gaboxadol to significantly augment alcohol, but not sucrose, self-administration at doses lacking overt sedative side effects (Fig. 2, top and middle). Although these results appear to implicate extrasynaptic $\alpha4\delta$ and/or $\alpha6\delta GABA_A$ receptors as regulators of the reinforcing properties of alcohol, other studies in mice[52-53] (but see [54]) found that gaboxadol *reduced* alcohol intake in both 2-bottle choice and drinking-in-the-dark procedures. Systematic studies are needed to draw firm conclusions about gaboxadol's ability to modify alcohol self-administration.

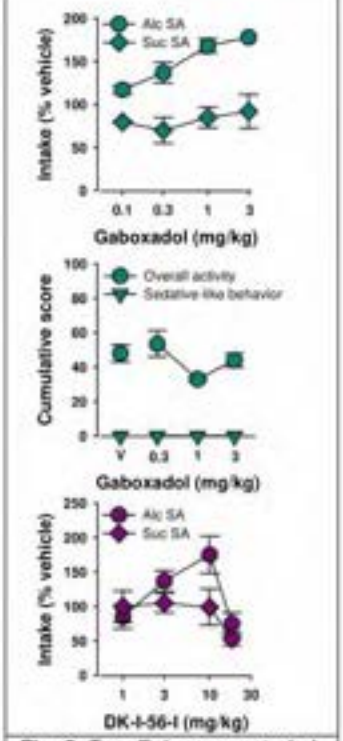


Fig. 2. Top: Enhancement of alcohol, but not sucrose, self-administration by gaboxadol. Middle: Gaboxadol does not alter overall activity or increase sedative-like behaviors. Bottom: DK-I-56-I also increases alcohol, but not sucrose, self-administration at 10 mg/kg. *N=3*.

Molecular/genetic studies have assessed more directly the role of these subunits in the reinforcing effects of alcohol. In that regard, δ knockout mice in a 2-bottle choice model show decreased preference and consumption of alcohol[55], an effect confirmed using RNAi techniques to decrease δ subunit mRNA and protein in the nucleus accumbens shell[56]. In a similar manner, Rewal and colleagues[57] used RNAi to decrease expression of the $\alpha4$ subunit in the nucleus accumbens core and shell and observed operant responding for alcohol to be reduced when this subunit was inhibited in the shell, but not the core. Generally, these results imply a potentially key role for the δ subunit, especially co-expressed in receptors with the $\alpha4$ subunit, in the reinforcing effects of alcohol. Possible specific contributions of $\alpha4\gamma$ and $\alpha6\gamma$, as well as $\alpha6\delta GABA_A$ receptors to alcohol reinforcement remain unknown, although our exciting preliminary data with the novel compound DK-I-56-I, an agonist at $\alpha6\gamma$ and $\alpha6\delta GABA_A$ receptors, show a marked increase only in alcohol self-administration across the doses tested (Fig. 2, bottom). This result is in line with observations of a significant association between polymorphisms in *GABRA6* (the gene encoding the $\alpha6$ subunit) and AUD across populations[25, 58].

Given the potential for $\alpha4\delta GABA_A$ receptors (at least) to facilitate alcohol's reinforcing effects, one might predict that these same receptors play a role in its subjective effects, as interoceptive effects may contribute to the initiation of drug taking in intermittent users and to the relapse process in drug abusers[59]. However, in monkeys and other lab animals trained to discriminate alcohol, neither gaboxadol nor GHB (agonist at $\alpha4\delta GABA_A$ receptors) produce alcohol-like effects up to doses that reliably decrease responding (Fig. 3, ref. [60-61]). Moreover, in rats trained to discriminate gaboxadol, alcohol fails to engender any substitution nor

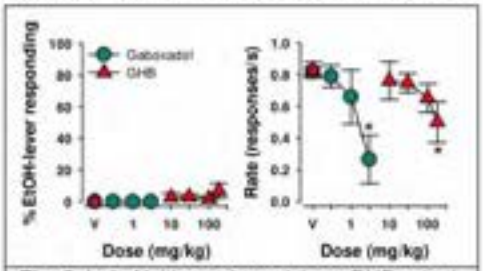


Fig. 3. Left: Neither gaboxadol nor GHB substitute for the DS effects of alcohol. Right: Both gaboxadol and GHB reliably decrease response rate in monkeys discriminating alcohol from vehicle (*N=4*). *: $p<0.05$ vs. V

does it enhance the DS effects of gaboxadol[62]. Finally, δ subunit knockout mice learn to discriminate alcohol similarly to wildtypes[63]. These results suggest that not only is there no overlap in the DS effects of alcohol, gaboxadol and GHB, but that δ subunits are not necessary for expression of the DS effects of alcohol. Furthermore, and consistent with the purported relationship between interoceptive effects and relapse, gaboxadol does not engender alcohol-seeking behavior in a reinstatement paradigm in mice[64]. However, possible roles of $\alpha4\gamma$ and $\alpha6\gamma GABA_A$ receptors in the DS and relapse-inducing effects of alcohol remain to be determined definitively, especially using pharmacological approaches.

2cii. Animals models of MAT – A novel approach to behavior therapy research: For AUD, pharmacotherapies typically are one of the last options to be offered to the patient; rather, behavioral therapies are the approach of choice[65-66]. However, human studies provide ample evidence that patient outcomes typically are improved when behavioral therapies are combined with adjunctive pharmacotherapies in a medication-assisted therapy (MAT) context (e.g., [67-68]). Moreover, implementation of MAT with different behavioral/pharmacological options allows clinicians to customize approaches to AUD treatment for individual patients[69]. Despite the apparent improved outcomes associated with MAT, the utility of this combination approach to assess therapeutic efficacy is understudied in the preclinical arena.

We recently have developed novel resurgence models of contingency management in rats[70] and monkeys. Contingency management, an approach in which patients are "paid" to abstain from alcohol drinking, is one of the most effective behavioral treatments for decreasing alcohol drinking (e.g., [71-72]). In our rat model, rats are trained to self-administer alcohol (Phase 1) and then are "paid" to withhold responses on the alcohol-paired lever (i.e., for every 10-s without a lever press, sweetened condensed milk is delivered; Phase 2). Under these conditions, alcohol seeking (as measured by lever presses to the alcohol-paired lever) decreases. When the behavioral treatment ends (i.e., milk delivery ceases) rats resume alcohol seeking, despite lever presses having no consequences (i.e., they "relapse"; Phase 3). We have found that resurgence of behavior is repeatable across cycles of self-administration, extinction and resurgence tests[70]. Importantly, when the behavioral therapy is combined with the AUD pharmacotherapy naltrexone, rats exhibit more rapid extinction of behavior in Phase 2 and fail to exhibit the resurgence of behavior in Phase 3. An effect that we also have observed, in a dose-dependent fashion, in monkeys (Fig. 4). In Aim 3, we will use the monkey model to assess the utility of GABAergic drugs, selected on the basis of their behavioral profiles (see section 4dii.), as adjunct pharmacotherapies in a MAT context.

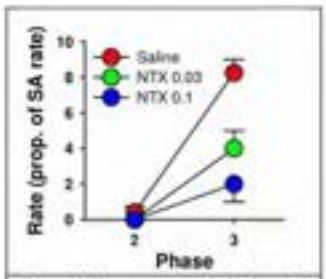


Fig. 4. Resurgence of alcohol seeking is dose-dependently attenuated by naltrexone in monkeys. (*N*=2).

3. INNOVATION

- Through our long-time collaboration with James Cook, Ph.D., Dept. of Chemistry, Univ. of Wisconsin-Milwaukee, we have access to first-in-kind GABAergic ligands targeting $\alpha4\gamma$, $\alpha6\gamma$, $\alpha4\delta$ and $\alpha6\delta GABA_A$ receptors for evaluation in our models of the abuse-related effects of alcohol. These novel compounds are the only selective ligands available for assessing the specific roles of these unique subtypes in the DS, reinforcing, and relapse-inducing effects of alcohol.
- The assessment of compound specific outcomes in both rhesus monkey and rat models is a powerful approach that will allow for determination of convergent results. Common effects of a compound across procedures and species may increase the likelihood of a positive outcome in human subjects.
- The approach of combining a highly effective behavioral therapy with a pharmacotherapy (i.e., MAT) to improve patient outcome is understudied in the preclinical arena. We have developed a novel resurgence-based animal model of contingency management therapy and will use this model to begin exploring the utility of adjunctive treatment with selective GABAergic ligands to improve clinically-relevant outcomes related to relapse.

4. APPROACH

4a. General methods:

4ai. Subjects and apparatus: Alcohol discrimination, self-administration, observation and resurgence experiments will be conducted with adult rhesus monkeys (*Macaca mulatta*) living in individual home cages. Each experiment will include both experienced and naïve subjects, allowing us to assess whether alcohol history *per se* results in functional adaptations in key $GABA_A$ subtypes. Subjects in the alcohol discrimination studies will

be habituated to a jacket and tether system (Lomir Biomedical). Monkeys will be provided with water and fed after the experimental session. Cages will be modified to accommodate custom-made operant conditioning panels containing response levers, drinking spouts (for self-administration and resurgence studies), stimulus lights and a food pellet dispenser and receptacle (Med Associates, Inc.) along with alcohol and water reservoirs.

In alcohol discrimination experiments involving intra-gastric drug administration, monkeys will be implanted with an indwelling gastric catheter using the surgical procedures described in ref. [73]. Catheters will be routed through a stainless steel tether attached to the monkeys' custom fitted nylon mesh jackets, and connected to a swivel mounted on the front of the cage. Catheters will be flushed daily with water. We have found that catheters implanted in this fashion will remain patent for several years. In these experiments, body weights will be maintained at approximately 90% of free-feeding values by adjusting access to food in the home cage.

Cue-induced reinstatement of alcohol/sucrose seeking and alcohol deprivation effect studies will be conducted with adult Wistar rats housed singly or in pairs, depending on the study, in standard shoebox cages. Rats will be provided with ad lib food and water. Reinstatement studies will be conducted in standard operant conditioning chambers equipped with response levers, stimulus lights, liquid reservoirs, and a syringe pump to deliver solutions to the reservoirs (Med Associates, Inc.). Alcohol deprivation effect studies will be conducted in the home cage by mounting volumetric drinking tubes to the top of the cage.

4aii. Blood alcohol levels: At selected points across the course of alcohol self-administration studies, venous blood will be collected from ketamine-anesthetized monkeys and analyzed with an Analox AM1 series analyzer (Analox Instruments USA Inc.) for determination of blood alcohol levels (BALs) as described in ref. [74]. BALs over 100 mg/dl are correlated with behavioral signs of intoxication[75]. Moreover, determination of BALs will facilitate extrapolation of our findings to humans because peak alcohol levels and clearance rates in macaques closely resemble those of humans[76].

4aiii. Alcohol discrimination: Our studies will use i.g. drug discrimination techniques to evaluate the contribution of $GABA_A$ receptor subtypes to the discriminative stimulus effects of alcohol. Monkeys will be trained to discriminate alcohol from water using procedures as described in ref. [18]. The i.g. route facilitates comparison of results obtained in drug discrimination and oral drug self-administration studies and minimizes potential problems of interpretation arising from pharmacokinetic differences associated with different routes of administration. Monkeys will be trained under a 30-response fixed-ratio (FR) schedule of food presentation (1 g, Bioserve) to respond differentially on the left and right levers depending on whether alcohol or water is administered. Daily sessions will consist of administration of either alcohol or water followed by a 120-min pretreatment period during which responding has no programmed consequences. This pretreatment interval corresponds to time to peak blood alcohol after administration of the training dose[18]. At the end of the pretreatment period, onset of stimulus lights will signal that the operant panel is active and that the FR schedule is in effect. The session will end after the completion of 30 FRs or 30 min, whichever occurs first. Training will continue until a criterion of five consecutive sessions in which at least 80% of the responses in the first ratio, as well as in the entire session, occur on the condition-appropriate lever is met. Monkeys will be trained to discriminate 2.0 g/kg alcohol based on the results of ref. [77], which showed optimal performance at this dose. Once stable discriminative control has developed, test sessions will be conducted once or twice per week, with training sessions on intervening days. During test sessions, 30 consecutive responses on either lever will produce food, and the session will end after completion of 30 FRs or 30 min. A single dose of a drug will be administered in a test session. We have successfully followed these procedures to establish i.g. alcohol discrimination in our monkeys (Fig. 3, ref. [18]).

Troubleshooting: In drug discrimination studies, problems typically arise as a lack of stimulus control and/or impairment of lever pressing by the training drug. Lack of stimulus control during training usually can be addressed by altering the training dose – we will do so if no evidence of discrimination is evident after 75 sessions. Regarding impairments in lever pressing, if monkeys are unable to complete response requirements following the training drug, we will titrate the training dose to a lower level until responding returns but with evidence of stimulus control intact. Individual differences in stimulus control sometimes arise, and if necessary, the FR and/or training dose can be tailored to an individual monkey's performance. Finally, duration of the pretreatment period easily can be varied to accommodate shorter or longer onsets of action of test drugs.

4aiv. Oral alcohol/sucrose self-administration: We will use oral self-administration procedures to assess the role of $GABA_A$ receptor subtypes in the reinforcing effects of alcohol. Monkeys will be induced to consume alcohol (4% w/v) using scheduled food pellet deliveries (i.e., schedule-induced polydipsia[78]) in 3-hr daily sessions. Schedule-induced polydipsia is an effective procedure for inducing consumption and has been used

successfully to establish operant responding maintained by alcohol at concentrations as high as 32% w/v (e.g., [79-80]). During the induction phase, white stimulus lights located above the spouts in the center of the operant panel will be lit indicating the start of the experimental session. Alcohol will be available from one spout, and will be signaled by illumination of red spout lights (i.e., alcohol-paired stimulus lights). Food pellets will be delivered to a receptacle located below the spouts in the center of the operant panel at fixed time intervals that result in the maximum consumption of the alcohol solution. We anticipate using a fixed time of 600 sec between food pellet deliveries (cf. [80]). The induction phase will continue until the monkeys reach a criterion level of intake, defined as drinking 150-250 ml within 90 min, corresponding to the volume necessary to self-administer a pharmacologically relevant alcohol dose of 1.5 g/kg, with a concentration of 4% w/v.

Following induction, monkeys will be trained to respond under a FR schedule of oral alcohol reinforcement as described in ref. [74]. Briefly, in the presence of a white light, completion of every 10th response (FR 10) will result in a change in illumination from white to red light and extension of the drinking spout. Depression of the drinking spout during extension will result in alcohol delivery. Fluid flow will continue for as long as the monkey displaces the drinking spout or for 30-s, whichever is shorter. The actual duration and volume of intake, within the constraints of the maximum duration of sipper extension, will be under the control of the subject. Between extensions of the drinking spout, all lights will be off briefly (<1 s), and responses will have no scheduled consequences. Daily self-administration sessions will last 3-h and food pellet delivery will be discontinued. The reinforcing effects of a range of concentrations of alcohol (0.5 – 16% w/v), as well as water, will be evaluated initially in each monkey. Each alcohol concentration (or water) will be made available for a minimum of five consecutive self-administration sessions and until stable intake is observed (i.e., no upward or downward trend in mls and/or g/kg consumed over a period of at least three consecutive days). We have successfully used these procedures to establish stable and robust alcohol self-administration in our monkeys (Figs. 1 and 2; ref. [18, 20, 35, 74]).

To determine behavioral selectivity of the effects of subtype-selective ligands, we will determine their capacity to modulate self-administration of a non-drug reinforcer in separate groups of male and female monkeys responding under a FR schedule of sucrose solution delivery. Training/testing procedures for these experiments will be as similar as possible to those described above for the induction and maintenance of alcohol self-administration, and we will vary the concentration of sucrose per delivery to develop performances that are as comparable as possible to those maintained by oral self-administration of alcohol (cf. [18, 20, 35]). The maintenance concentration of sucrose will be chosen to be that which produces stable intakes comparable to alcohol.

Trouble-shooting: During induction of alcohol self-administration, it is possible that some monkeys will not consume large quantities of alcohol, despite food pellet deliveries. If this occurs, we will reduce the concentration of alcohol as needed to make it more palatable to the animal and/or increase the frequency of food pellet delivery (i.e., reduce the fixed time interval to 300 sec) as required.

4av. Observable behavior: We will establish quantitative profiles for the behavioral effects of GABAergic ligands, both in the presence and absence of alcohol, to determine the doses of these drugs that induce side effects that could impact the suitability of these ligands as potential therapeutics (e.g., Fig. 2, middle). Our strategy is to focus on behavioral consequences that could adversely affect compliance with agonist-type therapies (e.g., behaviors indicative of sedative and ataxic effects) and antagonist-type therapies (e.g., behaviors indicative of anxiogenic effects). After self-administration sessions, alcohol and sucrose self-administration monkeys will be observed and their behavior scored for a 5-min sampling period (Fig. 2; cf. [18, 20, 35]). Comparing the effects of the GABAergic ligands in the alcohol-maintained animals to their effects in the sucrose-maintained animals allows us to evaluate the effects of the pretreatment alone (e.g., in the sucrose-maintained animals) and to assess the interaction between alcohol and the pretreatment drug (e.g., in the alcohol-maintained animals). Observers will be blind to all test conditions and trained in the use of the behavioral scoring instrument developed in our previous studies[18, 20, 27, 35]. Each observer will undergo at least 20 hrs of training until they reach a >90% reliability criterion based on percent agreement scores. Our scoring system includes 19 categories encompassing a wide range of species-typical and drug-induced behaviors including locomotion, object manipulation, foraging, self-grooming, scratching, stereotypic behavior, ataxia, rest/sleep posture, moderate and deep sedation. These behavioral categories have proven to be useful for the characterization of the sedative and motoric effects of GABAergic ligands[27, 81] as well as for the detection of possible anxiogenic effects in monkeys[35]. We will expand our list of behavioral categories as necessary to incorporate new behaviors that may be induced or affected by alcohol and/or test drugs. Each 5-min sampling period will be subdivided into twenty 15-s intervals, and frequency scores will be calculated as the proportion of 15-s intervals in which a particular behavior occurs.

Trouble-shooting: Although we habituate monkeys to the presence of an observer, it remains a possibility that the observer's presence will disturb the monkeys' behavior. If that appears to be the case, we will use video cameras to record the animals' behavior. It also is possible that a single 5-min sampling period may be insufficient to capture an accurate picture of the monkeys' behavior. In this case, we would increase sampling to four separate time points: before administration of the pretreatment drug to obtain baseline behavioral measures and after the first, second and third hours of self-administration.

4avi. Cue-induced alcohol/sucrose seeking: We will use cue-induced reinstatement procedures to assess the role of $GABA_A$ receptor subtypes in modulation of alcohol-seeking behavior. Rats will be trained to self-administer alcohol using a standard sucrose-fading procedure[82-83]. Briefly, in the presence of a white light, completion of every FR 2 on the active lever will result in the delivery of 0.1 ml of solution and activation of a flashing, amber cue-light located in the center of the operant conditioning panel (i.e., the alcohol-paired cue). A 1-s time-out period will follow during which all lights will be off and responses have no scheduled consequences. At the start of each self-administration session, the equivalent of one delivery of solution will be available in the reservoir below the active lever. This amount of alcohol is not pharmacologically relevant and functions to provide additional odor/taste cues[84-85]. Self-administration training will continue until subjects self-administer a 2% sucrose/15% alcohol w/v solution. Sessions will occur 5 days per week, last 30 minutes, and continue until stability criteria (3 consecutive days with a self-administered dose > 0.5 g/kg with no upward or downward trends; cf. [86]) are met. Extinction training will follow and continue until responding declines and stabilizes at the *a priori* criterion of ≤ 10% of self-administration baseline. During extinction sessions, the light above the active lever will be illuminated but lever presses will have no scheduled consequences. Cue-induced reinstatement tests will follow in which the conditions will be the same as for self-administration, except lever presses will result only in the activation of the cue-light with no solution delivered. We have used these procedures successfully to evaluate the effects of GABAergic ligands on stable and repeatable cue-induced alcohol seeking[36].

To determine selectivity of the effects of the $GABA_A$ ligands, we will determine their capacity to modulate cue-induced sucrose seeking in separate groups of rats responding under a schedule of sucrose solution delivery. Training/testing procedures for these experiments will be as similar as possible to those described above for alcohol-seeking behavior, and we will vary the concentration of sucrose for self-administration to develop performances that are as comparable as possible to those maintained by self-administration of alcohol. The maintenance concentration of sucrose will be chosen to be that which produces stable intakes comparable to alcohol.

Trouble-shooting: It is possible that some rats will not consume criterion quantities of alcohol. If this should happen, we can reduce the concentration of alcohol/delivery. Previously, we have shown that the effects of GABAergic ligands do not differ in rats administering 2% sucrose/10% or 15% alcohol w/v solutions[36].

4avii. Alcohol deprivation effect (ADE): We will use ADE procedures to assess the role of $GABA_A$ receptor subtypes in relapse-like drinking. The ADE is characterized as a transient increase in alcohol consumption following a period of abstinence after extended alcohol exposure (Fig. 1 bottom; e.g., [87-89]). This increase typically reflects a shift in preference to higher alcohol concentrations, rather than a simple increase in volume[89]. Following habituation, rats will have continuous access to drinking tubes on their home cage containing water, 5%, 10%, and 20% (v/v) alcohol solutions. Weekly, the positions of the tubes will be shifted randomly to avoid development of position biases. This initial exposure period will last 8 weeks. Subsequently, a 2 week long deprivation period will be imposed during which rats have access to water only. Following the deprivation period, the rats will again be given access to the alcohol solutions and water. The rats will undergo repeated exposure/deprivation cycles in which the period of access to alcohol will be varied between 4-8 weeks, but the abstinence period kept constant at 2 weeks. We have used these procedures successfully to evaluate the effects of GABAergic ligands on robust and repeatable relapse-like drinking in rats (Fig. 1 top; cf. [37]).

Trouble-shooting: It is possible that some rats will not demonstrate the ADE. However, we have chosen Wistar rats specifically because they demonstrate a robust ADE[89]. Given the nature of the procedure, though, we are able to alter the length of access to alcohol, as well as the length of the deprivation period. If necessary, we will adjust these periods to maximize expression of the ADE.

4aviii. Resurgence: To model contingency management, MAT, and, ultimately, relapse that may occur following the end of an intervention, we will use our novel resurgence model[70] in Aim 3. Resurgence studies will be initiated in the alcohol self-administration groups upon completion of Aim 1 studies. Our model is comprised of 3 phases: alcohol self-administration (Phase 1), contingency management/behavioral therapy (Phase 2), and resurgence (Phase 3). In Phase 1, monkeys will self-administer alcohol (4% w/v) under conditions described in

section 4aiv with the following modifications: session length will be decreased to 1-hr and FR requirements will be increased to 50. As resurgence data are plotted as a function of response rate in Phase 1, these procedural changes are designed to increase response rates in the monkeys and, thus, the likelihood of detecting an effect of our interventions in Phases 2 and 3. Once self-administration is stable under these conditions, Phase 2 will be initiated. In this phase, alcohol will no longer be available and contingency management will be modeled by "paying" the monkey one sucrose pellet for every 60-s that responding on the alcohol-paired lever is withheld. This phase will continue for 8 days during which behavior is expected to decrease to low, stable levels. In Phase 3, alcohol will remain unavailable but delivery of sucrose pellets also will be suspended. Resurgence, as indicated by an increase in responding on the alcohol-paired lever, will be measured for 5 days.

Trouble-shooting: Although unlikely given their history, it is possible that some monkeys will not maintain self-administration under the proposed conditions for Phase 1. We can adjust schedule parameters (e.g., FR requirement, duration of sipper extension) to achieve high, stable levels of responding. We acknowledge that these parameters result in moderate alcohol doses in a majority of animals (although some do achieve intakes mirroring heavy drinking levels, cf. [90]) and that this may limit the translational relevance to moderate drinking. Should we observe the anticipated positive outcomes (and if time and resources allow), we will further adjust parameters in an attempt to increase alcohol intake levels and reevaluate the therapies. Potentially, some monkeys will not extinguish lever pressing in Phase 2. If this occurs, we have options to adjust aspects of the pellet delivery schedule (# of pellets delivered, interval between deliveries) to ensure that we achieve low and stable rates of responding. However, any adjustments must be constrained so that the schedule of pellet delivery in Phase 2 is at least 4x as frequent as the delivery of alcohol in Phase 1. This 1:4 ratio of reinforcer delivery in Phases 1 and 2, respectively, has reliably produced resurgence in subjects across species and reinforcers[91-92].

4aix. Sex as a relevant biological variable, sample size and analysis strategies:

4aix*a*. Alcohol discrimination: To our knowledge, there is no strong evidence of gender differences in the subjective/DS effects of alcohol in humans. In monkeys, under the training dose we use, there are no reported sex differences in the substitution profile of GABAergic ligands[77]. Moreover, menstrual cycle does not appear to influence sensitivity to the DS effects of alcohol or GABAergic ligands under these conditions[93]. For these reasons, we propose 1 group of alcohol discrimination monkeys comprised of both male and female monkeys. For DS studies, effect sizes are relatively large (typically >0.80) due to the primary dependent measure (% drug-lever responding) reflecting a scale of 0 to 100%, with an effect based on training the subject to discriminate drug from saline to a high degree of accuracy. Combined with historical lack of sex-dependent effects in alcohol-trained monkeys, we will use a sample size of N=6 that will include half of each sex (with 4 doses and vehicle, n=6 will require power of f= 0.397 to detect significant differences at p≤0.05). Each subject will receive all test and control conditions whenever possible. This design, in which each subject serves as its own control, permits scientifically meaningful results to be obtained with fewer animals than would be required using other approaches. Repeated measures ANOVA will be used to evaluate statistical reliability of effects (% alcohol-lever responding, response rate). Multiple comparisons will be assessed using *a priori* Bonferroni t-tests as applicable. Although beyond the scope of the present project, with n=3 for each sex, we should be able to observe trends to explore in follow-up studies.

4aix*b*. Oral self-administration: Historically, men tend to drink larger amounts of alcohol than women (for review [94]); an observation that is paralleled in rhesus monkeys where males drink significantly more ethanol than females[80]. However, the incidence of alcohol use, high-risk drinking and AUD is increasing among women and other subgroups[1]. We propose to use 2 groups of alcohol self-administration monkeys – 1 male, 1 female – to explicitly evaluate the role of sex in observed effects. In monkeys and possibly humans, menstrual cycle has been shown to have modest, but statistically significant, effects on alcohol drinking[95]. We propose to track the onset and offset of menses 7 days per week by training female monkeys to present for vaginal swabs. Although we will not obtain conclusive data regarding hormone levels, by tracking menses onset/offset, we will be able to conduct exploratory data analyses to evaluate the extent to which self-administration is altered in relation to menstruation (days 1-5), mid-cycle (days 12-16), and late luteal (last 5 days) phases. Parsing the menstrual cycle in this manner has been shown to be sensitive to phase-dependent differences in alcohol intake in monkeys[95]. We also propose male and female groups for sucrose self-administration studies. We will monitor menstrual cycle in sucrose females as described. For direct statistical comparisons of alcohol drinking parameters between females and males, prior reports of sex differences in monkeys appear to be most robust with BALs,

with total consumption being less robust (e.g., Cohen's *d* = 1.60 vs. 1.07, respectively; [80]), translating into relatively "large" effect sizes, by convention. For a mixed factor ANOVA with sex as the between factor and concentration as the within (4 concentrations and vehicle control), an effect size (f) of 0.50 would require a total sample size of 12 to detect sex X concentration interactions (power [1-β]=0.95, nonsphericity correction [ε]= 1.0). Based on these estimates and the extant literature, we will use n=6 females and n=6 males in each of the self-administration groups. Each subject will receive all test and control conditions whenever possible. Mixed design ANOVAs (between factor: sex; within factor: dose) will be used to evaluate statistical reliability of effects on alcohol intake (e.g., mls, g/kg, BAL). Multiple comparisons will be assessed using *a priori* Bonferroni t-tests as applicable. Only planned comparisons will be used to compare across alcohol and sucrose groups.

4aix*c*. Observation: Observations are conducted in all of the alcohol and sucrose self-administration monkeys. Thus, we will be able to determine the extent to which sex plays a role in the observable effects/side effect profiles of selective GABAergic compounds. For each drug/behavior, we will follow the analysis strategy outlined for oral self-administration experiments.

4aix*d*. Cue-induced reinstatement: Although only limited data exist for human subjects, there is the suggestion that males and females may differ in alcohol cue reactivity, especially after low-to-moderate alcohol exposure[96]. In rats, females consistently demonstrate greater cue-induced alcohol seeking compared to males [97-99]. However, circulating gonadal hormones appear to have little effect on cue-induced alcohol seeking[100]. For each drug to be studied, we propose to use 2 groups of alcohol reinstatement rats (1 male, 1 female) to evaluate the role of sex in cue-induced alcohol seeking. We also propose separate male and female groups for sucrose reinstatement studies. Based on our previous study[36], effect sizes (f) ranged from 0.45 to 0.69. This resulted in power (1-β) of, minimally, 0.78. These parameters were based on N=8-10 for within-subjects analyses, and changing this to a mixed design, we calculate that we will have sufficient power (1-β ≈ 0.8) with 10 rats per group (i.e., 10 rats per sex, multiple pre-treatments).

4aix*e*. Alcohol deprivation effect: When assessed in rodent studies of the ADE, sex differences in relapse-like drinking are not evident[101-102]. Very little is known about ADE effects in humans. A single study[103] has examined motivation to self-administer alcohol after an abstinence period in heavy drinkers and found that females tended to work harder for alcohol. As the evidence for sex differences is equivocal, we propose to include even numbers of males and females in each group. Each drug will be studied in a different group of rats. Previous sample sizes for ADE studies are typically n>10, and in studies[37] with n=14, we observed a significant ADE effect ($p<0.05$) with an effect size (f) of 0.40. This resulted in power (1-β) of 0.83. This sample size is sufficient to detect significance at f= 0.30, therefore we will use this sample size (consisting of even numbers of male and female rats) for our planned studies.

4aix*f*. Resurgence: Resurgence studies will be conducted in the alcohol self-administration groups once they complete Aim 1 studies. As such, we will be able to determine the extent to which sex influences outcomes associated with MAT approaches. Although available data suggest that contingency management is equally effective in both sexes[104], it is unclear whether sex influences the outcome of combination/MAT approaches. Mixed design ANOVAs (between factor: sex; within factor: dose) will be used to evaluate statistical reliability of effects on Phase 1 (e.g., mls, g/kg), Phase 2 (rate on final day), and Phase 3 (rate on resurgence) variables. Multiple comparisons will be assessed using *a priori* Bonferroni t-tests as applicable.

4b. AIM 1: γ- vs. δ-containing α4 and α6GABA$_A$ receptors in the abuse-related effects of alcohol

4bi. Hypothesis: We hypothesize that δ-containing GABA$_A$ receptors are key facilitators of the reinforcing but not DS or relapse-inducing effects of alcohol. The specific contributions of α4δ, α6δ, α4γ, and α6γGABA$_A$ receptors, though, are unknown.

4bii. Experimental plan: We will evaluate a series of γ- vs. δ-selective α4 and/or α6GABA$_A$ ligands in monkeys trained to either discriminate intra-gastric alcohol from water or to orally self-administer alcohol, as well as in rats trained in the cue reinstatement procedure or undergoing cycles of the ADE procedure. Initially, to obtain a general understanding of the role of extrasynaptic vs. synaptic α4/α6GABA$_A$ receptors in the abuse-related effects of alcohol, we will compare the effects of gaboxadol, Thio-4-PIOL, and XHe-III-74 in our models. Gaboxadol (0.1-1 mg/kg) is a partial agonist that acts selectively at extrasynaptic α4/α6δGABA$_A$ receptors[48-49] and enhanced alcohol's reinforcing effects (Fig. 2). Compared to gaboxadol, Thio-4-PIOL (1-10 mg/kg) also exhibits selectivity but lower efficacy at α4δ/α6δ GABA$_A$ receptors[105]. XHe-III-74 exhibits functional selectivity for α4γ/α6γGABA$_A$ receptors, i.e, extrasynaptic *and* synaptic receptors (Fig. 5, next page), but does not modulate

α4δGABA$_A$ receptors[106]. Like alcohol, XHe-III-74 (1-30 mg/kg) has been shown to produce ataxia and/or muscle relaxation in mice[107]. If all three ligands produce similar outcomes in a given procedure, we would interpret this to mean that both extrasynaptic and synaptic GABA$_A$ receptors are modulators of the effect. This conclusion would be strengthened if the outcomes were efficacy dependent (i.e., XHe-III-74>gaboxadol>Thio-4-PIOL). If, however, XHe-III-74 is without effect, we would conclude that it is the extrasynaptic δ-containing GABA$_A$ receptors that are mediating the effect.

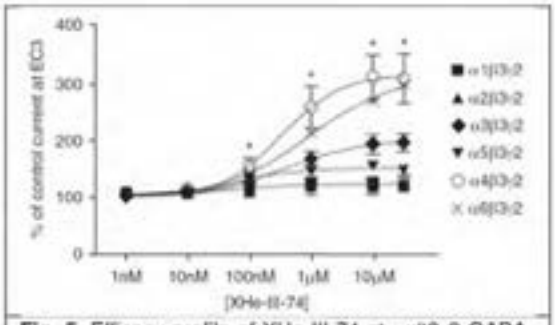


Fig. 5. Efficacy profile of XHe-III-74 at αxβ3γ2 GABA$_A$ receptors. ***XHe-III-74 does not modulate α4δ GABA$_A$ receptors.*** Data from ref. 106.

Additional ligands will be used to pharmacologically interrogate specific extrasynaptic vs. synaptic GABA$_A$ receptors and comparisons will be made with the less specific compounds described above. To target α4δ receptors, we will use γ-hydroxybutyric acid (or GHB; 30-300 mg/kg) which recently has been shown in vitro to be an agonist at this subtype[108]. GHB produces behavioral effects in nonhuman primates that are similar to alcohol (e.g., motor impairment, muscle relaxation, reinforcing effects; [109-110]). If GHB shares effects similar to that of gaboxadol (and perhaps THIO-4-PIOL), we would conclude that α4δ receptors, but not α6δ receptors, are key mediators of the effect. If GHB's effects do not resemble gaboxadol's, then extrasynaptic α6δ receptors are likely more important.

To target α6GABA$_A$ receptors, we will use two ligands: DK-I-56-I and Cook Compound 6. These ligands are deuterated and have improved metabolic stability and pharmacokinetics over N-hetero analogs[111]. DK-I-56-I (1-10 mg/kg) has been shown to be functionally selective for α6GABA$_A$ receptors[112], regardless of whether they contain the δ or γ subunit (Fig. 6), and when given systemically, enhanced alcohol self-administration in monkeys (Fig. 2). In vitro, Cook Compound 6 is functionally-selective for α6γGABA$_A$ receptors (Fig. 7)[113]. When given in vivo, compound 6 (1-18 mg/kg) attenuated the central effects of capsaicin in an animal model of migraine[114] and rescued methamphetamine-induced disruptions in prepulse inhibition of a startle response[115]. Because DK-I-56-I enhanced alcohol self-administration, we hypothesize that α6GABA$_A$ receptors modulate alcohol's reinforcing effects. If Compound 6 similarly modulates self-administration, we can conclude that it is α6γ receptors that drive the enhancement. Importantly, if Compound 6 is without effect, this would point to a key role for α6δ receptors.

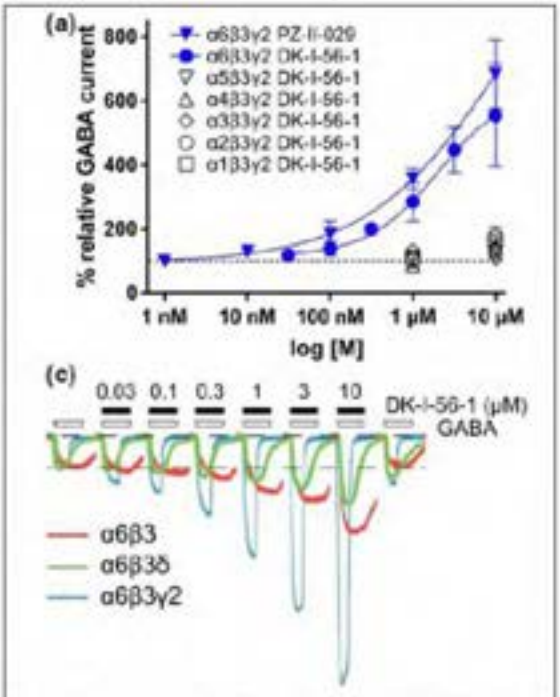


Fig. 6. Efficacy profile of DK-I-56-I at αxβ3γ2 and α6β3δ GABA$_A$ receptors. ***DK-I-56-I does not modulate α4γ GABA$_A$ receptors.*** Data from ref. 112.

Finally, to strengthen any conclusions regarding mechanisms when a positive result is obtained, we will conduct antagonism studies with the nonselective benzodiazepine antagonist flumazenil (note, no antagonists exist currently that are specific for γ- versus δ-containing α4 and α6GABA$_A$ receptors). In primates and rodents, flumazenil inhibits diazepam-sensitive GABA$_A$ receptors at low doses (e.g., 0.3 mg/kg) and diazepam-insensitive receptors (i.e., α4 and α6GABA$_A$ receptors) at high doses (e.g., 30 mg/kg)[116]. If the observed effect is mediated by α4 and/or α6GABA$_A$ receptors, we would expect it to be attenuated only by doses of flumazenil ≥30 mg/kg.

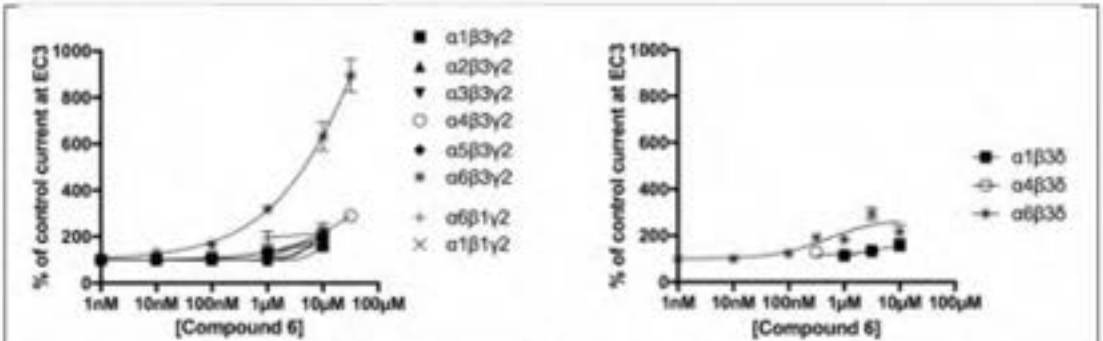


Fig. 7. Efficacy profile of Compound 6 at αxβ3γ2 and αxβ3δ GABA$_A$ receptors. Data from ref. 113 and unpublished.

Note, too, that the majority of the compounds are provided via our collaboration with James M. Cook. The ligands described above are the best pharmacological tools available currently to study the role of γ- and δ-containing α4 and α6$GABA_A$ receptors in the behavioral effects of alcohol. Synthesis of novel GABAergic ligands is an integral part of Dr. Cook's medicinal chemistry program, and our studies will be extended to include other compounds with improved selectivity and efficacy profiles as they are developed by Dr. Cook.

In general, we will determine initially the extent to which the ligands mimic the effects of alcohol (i.e., engender alcohol-like DS effects, maintain self-administration, induce alcohol seeking). After which, we will begin agonist combination studies by administering the ligands as pretreatments before determination of the alcohol dose-/concentration-response function. In discrimination and reinstatement, doses of the ligands will be studied in single day tests. In self-administration, a dose will be evaluated for a minimum of 5 consecutive days and until stable intake is observed. In ADE studies, a dose will be evaluated for 5 consecutive days (i.e., the last 2 days of an abstinence period and the first 3 days of alcohol re-exposure). Between tests with different doses of the agonists, the monkeys will be returned to baseline training for a period of 1 to 5 days depending on the procedure. In rats, operant self-administration/baseline drinking will be re-established for a period of 10 days to 8 weeks, depending on the procedure. Test sessions will occur only if baseline performance meets the test criteria for each procedure. Regardless of procedure, all doses of an agonist will be studied before moving to another ligand. In the context of some procedures, blood samples will be collected after test sessions to determine BALs. This information will permit us to determine whether observed changes are accompanied by changes in BALs. If a particular $GABA_A$ subtype contributes to the observed effects of alcohol, we would expect to see ligands targeting this subtype to both mimic and modulate the DS, reinforcing and relapse-inducing effects of alcohol.

Trouble-shooting: In monkey studies, we will administer the ligands via i.g. catheters or a sucrose solution in a water bottle. The sucrose will be used to mask any unpleasant tastes associated with the test compounds. We do not anticipate that the sucrose solution alone will alter behavior (i.e., intake of alcohol during self-administration sessions is similar regardless of whether animals receive a pretreatment with unadulterated sucrose solution). However, we will determine empirically the maximum volume of sucrose solution an animal can ingest before it begins to affect subsequent self-administration in pilot studies. Alcohol self-administration levels following sucrose vehicle pretreatment will be compared directly to self-administration levels in the absence of vehicle pretreatment. Also, if we find a compound that is inactive when administered orally, we will administer at least two doses of the compound using a route of administration by which it has demonstrated activity.

4c. AIM 2: GABAergic ligands – Specificity of effects

4ci. Hypothesis: Given the postulated role for δ-containing $GABA_A$ receptors as specific facilitators of alcohol self-administration, we do not expect δ-selective ligands to modulate sucrose self-administration or seeking. In contrast, we do expect these ligands to engender observable effects in monkeys similar to alcohol (e.g., ataxia, sedative-like effects). The specific effects of α4δ, α6δ, α4γ, and α6γ$GABA_A$-selective ligands are unknown, which will represent an important contribution to the literature on $GABA_A$ pharmacology on its own.

4cii. Experimental plan: We will compare the effects of drug pretreatments in the experiments described in Section 4bii with those in male and female monkeys responding under an identical FR schedule of sucrose solution delivery (see section 4aiv), and in male and female rats trained in cue-induced sucrose reinstatement procedures (see section 4avi). Once suitable baseline performances are established, we will determine the effects of selective $GABA_A$ ligands on self-administration of a concentration of sucrose that maintains behavior comparable to that of the "peak" alcohol concentration, and on cue-induced sucrose-seeking behavior. Initially, we will evaluate the effects of doses of GABAergic ligands that reliably modify self-administration of alcohol or modulate alcohol-seeking behavior, using testing procedures as described above. If a selective ligand does not reliably alter sucrose self-administration at doses that modulate alcohol self-administration, or modulate sucrose seeking at doses that alter alcohol seeking, the dose of the GABAergic ligand will be gradually increased until reliable increases/decreases in behavior are observed. The ability of $GABA_A$ ligands to modify alcohol self-administration/seeking at doses that do not similarly alter sucrose self-administration/seeking would suggest that the modulation of alcohol's effects is not due simply to a generalized disruption of behavior. Comparisons of the doses of GABAergic ligands that reliably change alcohol self-administration/seeking with those that alter sucrose self-administration/seeking also will provide information regarding a compound's potential therapeutic dose range. We also will establish quantitative profiles in monkeys for the behavioral effects of selective $GABA_A$ ligands, both in the presence and absence of alcohol, to determine the doses of these drugs that induce side

effects that could impact the suitability of these ligands as potential therapeutics (see section 4av). Comparing the effects of the GABAergic ligands in the alcohol-maintained monkeys to their effects in the sucrose-maintained monkeys allows us to evaluate the effects of the pretreatment alone (e.g., in the sucrose animals) and to assess the interaction between alcohol and the pretreatment drug (e.g., in the alcohol animals).

4d. AIM 3: GABAergic ligands as pharmacological adjuncts to behavioral therapy (i.e., MAT)

4di. Hypothesis: We hypothesize that the addition of GABAergic ligands to behavioral therapies will result in improved therapeutic outcomes as evidenced by accelerated extinction of drug-taking behavior and reduced relapse-like effects.

4dii. Experimental plan: We will evaluate the utility of selective GABAergic ligands with favorable side effect profiles (identified in previous studies from our laboratory) to serve as co-therapies in a model of MAT. These studies will make use of a novel resurgence model of contingency management that we recently developed (see section 4aviii). We will initiate pharmacological treatments at the start of Phase 2 (contingency management/behavioral therapy) and continue drug administration into Phase 3 (discontinuation of behavioral therapy) for 5 days. Pharmacological treatment then will be discontinued for an additional 5 days, before alcohol is again made available. Compared to discontinuing both Phase 2 interventions at once or delivering interventions serially (i.e., behavioral intervention in Phase 2 and pharmacological intervention in Phase 3), our strategy of discontinuing therapeutic interventions in a tapered manner has shown to produce the best outcome in that subjects do not exhibit resurgence of alcohol seeking behavior upon cessation of all treatment[70]. Monkeys will undergo repeated cycles of the resurgence procedure to evaluate different doses and drugs (cf. Fig. 4).

Our choice of GABAergic ligands to study initially has been guided by strategies used to treat addiction to other drugs of abuse. One therapeutic approach that has proven effective is agonist maintenance (e.g., methadone for heroin dependence), and a pharmacotherapy of this type would be expected to mimic at least in part the pharmacology of alcohol. Another therapeutic approach is based on pharmacological antagonism. A desirable medication of this type would be expected to decrease or eliminate the pharmacological effects of alcohol. As described in section 2bii and 2biii, α2/3GABA$_A$ agonists and α5GABA$_A$ inverse agonists possess profiles that match these two descriptions, respectively. In this regard, α2/3GABA$_A$ agonists share DS effects with alcohol and selectively modulate alcohol self-administration; α5GABA$_A$ inverse agonists act as pharmacological antagonists of multiple abuse-related effects of alcohol. Thus, we propose to initially evaluate the α2/3GABA$_A$ agonists MP-III-080 (1-10 mg/kg; [117-118]) and YT-III-31 (0.1-3 mg/kg; [18, 30]), and the α5GABA$_A$ inverse agonist RY-23 (0.03-1 mg/kg; [34]). The effects of GABAergic drugs will be compared to that of the AUD pharmacotherapy naltrexone that we have shown to improve outcomes in our resurgence model (cf. Fig. 4). In a pilot resurgence study, we obtained exciting new findings showing that RY-23 both facilitates the rate of extinction in Phase 2 and prevents the resurgence of alcohol seeking in Phase 3 (Fig. 8).

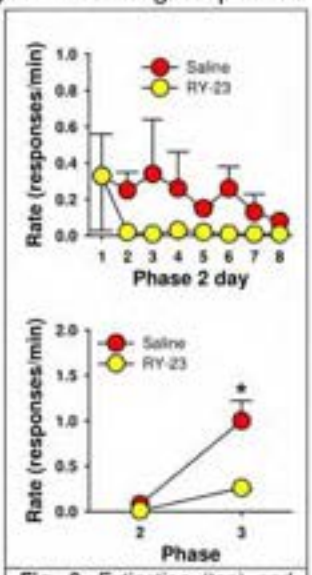


Fig. 8. Extinction (top) and resurgence of alcohol seeking (bottom) are attenuated by the α5GABA$_A$ inverse agonist RY-23. (N=4).

4e. Rigor, reproducibility and timeline: Several strategic aspects contribute to enhanced rigor and reproducibility. These include 1) inclusion of both male and female subjects, 2) adequate sample sizes as determined via power analysis, 3) use of within-subjects design and recapturing of baseline performance across the course of a study, 4) evaluation of full dose-response functions in which dose administration is counter-balanced between animals, and 5) use of observers trained to an inter-observer reliability criterion and blinded to experimental conditions.

	Year 1	Year 2	Year 3	Year 4	Year 5
AIM 1	1. Purchase SA females 2. Train SA procedure 3. Initiate Aim 1 (SA) 4. Initiate AR studies	1. Continue Aim 1 studies (SA) 2. Purchase females (AD) 3. Train AD procedure 4. Continue AR studies	1. Complete Aim 1 studies (SA) 2. Initiate Aim 1 studies (AD) 3. Complete AR studies	1. Continue Aim 1 studies (AD) 2. Initiate ADE studies	1. Complete Aim 1 studies (AD) 2. Complete ADE studies
AIM 2	1. Observation (SA) 2. Initiate SR studies	1. Observation studies (SA) 2. Continue SR studies	1. Observation (SA) 2. Purchase females (SS) 3. Complete SR studies	1. Train SS procedure 2. Initiate Aim 2 studies (SS) 3. Observation (SS)	1. Complete Aim 2 studies (SS) 2. Observation (SS)
AIM 3			1. Transition SA groups to RS procedure	1. Initiate Aim 3 studies (RS)	1. Complete Aim 3 studies (RS)

SA: Alc self-admin; AD: Alc discr; SS: Suc self-admin; RS: Resurgence; AR: Alc reinstate; SR: Suc reinstate ADE: Alc deprivation effect

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002
Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

VERTEBRATE ANIMALS

Rhesus monkeys in this application will be housed in the Redacted by agreement Redacted by agreement. Wistar rats will be housed in the vivarium of the Redacted by agreement

1. Description of procedures. Our proposed research will investigate the role of specific GABAergic mechanisms in the effects of alcohol that promote its abuse using nonhuman primate and rodent models. To that end, we will evaluate the ability of ligands selective for γ- and δ-containing α4 and α6$GABA_A$ receptors to modulate 1) the DS effects of alcohol in rhesus monkeys trained to discriminate intragastrically-administered alcohol from vehicle, 2) oral self-administration of alcohol vs. sucrose in rhesus monkeys, and 3) cue-induced reinstatement of alcohol vs. sucrose, as well as the alcohol deprivation effect (ADE) in rats. Additionally, using a novel resurgence model, we will identify therapeutic conditions under which selective GABAergic ligands (initially α2/3$GABA_A$ receptor agonists and α5$GABA_A$ receptor inverse agonists) may have utility as pharmacological adjuncts to more commonly used behavioral therapies and improve patient outcomes. These studies will provide needed information about specific $GABA_A$ mechanisms that may underlie the addictive effects of alcohol and will aid identification of GABAergic ligands for the pharmacological management of alcohol abuse and relapse.

A maximum of 30 monkeys (15 males and 15 females) and 84 rats (42 males and 42 females) per year will be maintained during the project period. The monkeys will be rhesus monkeys obtained from domestic breeding colonies and will be pre-screened for pathogens, including Herpes simian B virus (Macacine herpesvirus 1). Newly acquired monkeys will be kept in a separate quarantine facility for at least 40 days before being introduced into the colony. Monkeys will be at least 5 years old and will weigh 5-8 kg at the beginning of the studies. The rats will be of the Wistar strain and obtained from Charles River, Inc.

Monkeys will live in individual stainless steel cages in a vivarium with regulated temperature, humidity, air exchange, and light-dark cycle. The cages will be sanitized at a minimum of once per 2 weeks, and absorbent bedding material in cage pans will be changed at least every other day. To promote socialization, cages are grouped together in colony rooms to permit visual, auditory and olfactory contact with other animals. Supervised technicians will be responsible for daily care and feeding of all monkeys. In general, monkeys will have unrestricted access to water. They will receive a nutritionally balanced diet consisting of standard monkey biscuits supplemented with fruits, grains, and vegetables. Monkeys are weighed at least once every two weeks and diets are adjusted based on both health considerations and the requirements of the experiments. The weights of the monkeys are recorded, and comparisons with average weights for age and sex can be obtained from databases maintained by the Center for Comparative Research (CCR, laboratory animal facilities).

Rats will be pair- or individually-housed depending on the study (i.e., reinstatement: paired; ADE: individual) in standard shoebox cages in a vivarium with regulated temperature, humidity, air exchange, and light-dark cycle (Note: rats will be housed under a reverse light-dark cycle and reinstatement studies will be conducted during the dark portion of the cycle; ADE studies occur around-the-clock). The cages/bedding are changed twice weekly. Supervised technicians will be responsible for daily care and feeding of all rats. Rats will have ad lib access to water and standard rat chow. Rats will be weighed once/week, at a minimum.

2. Justifications.

Alcohol use disorders are clearly significant problems in our society. A critical step in the effective management of alcohol addiction is to establish definitive data regarding factors controlling the abuse-related effects of alcohol, and to use this information to facilitate development of effective therapeutic strategies. Rhesus macaques and rats are ideally suited for preclinical research on these questions. These species have been used in behavioral pharmacology research for over 50 years and have provided valid and reliable models of different aspects of alcohol addiction in humans. Because of the extensive use of both rats (of many strains) and macaques in alcohol research, there is a large body of scientific information which will provide indispensable comparative information for proper interpretation of our research and for its application to the development of medications to treat alcohol abuse and dependence. The proposed work cannot be conducted in humans because of the addictive and/or experimental nature of the drugs under study. The research also cannot be conducted using tissue samples or biological material because a primary goal of the research is to determine how drugs alter and control behavior in models predictive of effects in humans. At this point in time, computer modeling does not

provide meaningful information for research of this type, since modeling depends largely on *a priori* information that is not available without first conducting the types of studies described in this application.

The number of monkeys and rats to use in behavioral experiments is a decision that involves a trade-off between using large numbers of valuable animals and assuring the reliability of data collected using smaller numbers of animals. The monkey experiments proposed in this application are designed to increase the reliability of data from small numbers by using, whenever possible, a within-subjects experimental design. This design, in which each animal serves as its own control, permits scientifically meaningful results to be obtained with fewer animals than would be required with other types of designs (e.g., an exclusively between-subjects approach). The rat experiments proposed in this application use a mixed-design approach where groups of animals receive all doses of a particular drug, but different groups are assigned to different drug treatments. This design is a compromise between the large numbers of rats necessary for an exclusively between-subjects design, and the length of time necessary to conduct exclusively within-subjects design experiments (i.e., the life span of a rat could not accommodate the testing of all possible doses in a given procedure). Importantly, in all instances, power analyses of previous studies from our laboratory and others have been used to determine appropriate sample sizes.

3. Minimization of pain and distress.

The research will be conducted with veterinary supervision from the University of Mississippi Medical Center (UMMC) Center for Comparative Research (CCR). The CCR is attended by a full-time veterinary and support staff, including two full-time veterinarians, four certified veterinary technicians, and a veterinary assistant. This staff is experienced in providing high quality care to laboratory animals, including monkeys. Monkeys, specifically, have been on-site at UMMC for more than two decades, and the veterinary staff is experienced and equipped to house and care for them. Rats have been on campus for a substantially longer period of time. Monkeys and rats will be housed in Redacted by agreement in rooms custom designed for this research. CCR and veterinary staff provide daily husbandry and veterinary care for animals that is consistent with the Guide for Care and Use of Laboratory Animals. Redacted by agreement, the attending veterinarian, is the Director of CCR and is Board Certified by the American College of Laboratory Animal Medicine. All animal care and experimental procedures are conducted in accordance with laws, regulations, and guidelines of the PHS Policy, Office of Laboratory Animal Welfare, United States Department of Agriculture, UMMC's Institutional Animal Care and Use Committee, and CCR institutional policies.

The CCR at UMMC has in place an active Environmental Enrichment Program to promote the humane care and psychological well-being of all experimental animals. All animals are housed in groups unless experimental design specifically requires individual housing in which case special exemption must be sought and approved by the IACUC. Depending on the species and the study requirements, singly-housed animals are given opportunities for socialization and cage structure and configuration is designed to maximize social opportunities. Enrichment is provided through play devices, foraging opportunities, special food items, structural and environmental enhancements, and positive human interaction. Devices available include foraging boards, puzzle feeders, toys, mirrors, and audiovisual devices.

The PI, laboratory personnel and animal technicians involved in the proposed research will have had instruction or demonstrated their competence in the care, use and handling of laboratory animals. We give assurance that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals. Discomfort will be limited to what is unavoidable in conducting the research. Clinical veterinarians in the CCR will be responsible for diagnosing and treating animals displaying signs of pain and distress and for medical evaluations as required.

Discomfort, distress, or pain may be associated with the intra-gastric catheter implantation for alcohol discrimination studies. To minimize these effects, surgeries will be conducted using strict aseptic techniques and anesthesia (inhaled isoflurane). Veterinary staff will be present throughout all surgeries to monitor vital signs and anesthesia during the procedure. Animals are initially sedated with ketamine (5-10 mg/kg, i.m.). Sensorcaine (0.25%) will be applied locally before suturing, carprofen (2-4 mg/kg, s.c. or p.o.) will be administered pre- and post-operatively for pain, and an antibiotic (usually Keflex, 20-25 mg/kg) will be administered to prevent infection. Following surgery, veterinary staff and research technicians will monitor monkeys continuously until they are

awake. Once awake, monkeys will be monitored every 15-30 min by personnel until they take and eat treats or fruit. Veterinary and research staff will continue to monitor animals on a daily basis post-surgery for a minimum of one week. Experimental sessions will not begin until animals are fully recovered from surgery, a minimum of 1-2 days.

In alcohol discrimination studies, monkeys receive food reinforcement. Stable daily performance is maintained by restricting access to food in the animal's living quarters. During initial training, body weights are reduced to approximately 90% of ad libitum values. Once subjects respond reliably under the schedule of food reinforcement, home-cage feeding is increased to the maximum allotment that can be given without resulting in degraded performances during experimental sessions. Fruits and vegetables also are given as supplements. Over the course of the experiments, we anticipate that body weights will be maintained at 90 – 95% ad libitum values. Adult rhesus monkeys can be maintained indefinitely at such weights with no untoward effects or risks to health.

Rats will be habituated to their cages and housing room prior to the onset of the studies. They will be handled routinely and habituated to the injection process in order to reduce the occurrence of stress.

Across all procedures, doses of test compounds used in our proposed studies are carefully selected to provide scientifically meaningful data and are not expected to induce significant toxicity or compromise the health of the subjects. All drugs and vehicle will be pharmaceutical grade, received directly from NIH/NIDA, or received after extensive purity analysis by the laboratory of our collaborator, Redacted by agreement Redacted by The primary effects of the compounds are expected to be temporary and reversible. Should untoward effects occur for any reason, however, experiments will be discontinued until the cause of the problem can be identified and resolved to minimize pain and distress.

A significant number of subjects in our studies will self-administer alcohol over the course of several months. These procedures are integral to the aims of this project. Based on previous research, we do not expect withdrawal symptoms with the access conditions we propose. However, should withdrawal signs be observed, we will discontinue alcohol self-administration by reducing the concentration gradually to avoid possible distress.

4. Euthanasia.

For monkeys, there is no planned euthanasia in these studies. However, it is possible that it may become necessary as a result of an illness or other non-experimental event. Otherwise, monkeys will be transferred to alternative protocols at the end of the proposed studies. In the event euthanasia is necessary, animals will be first sedated with Telazol (tiletamine HCl/zolazepam HCl) 5 mg/kg i.m. followed by a lethal i.v. injection of a pentobarbital solution. Secondary confirmation is achieved via bilateral pneumothorax followed by necropsy. This method of euthanasia is consistent with the recommendations of the panel on euthanasia of the American Veterinary Medical Association (AVMA).

Non-experimental endpoint decisions are based on: 1) loss of 25% body weight from the maximum body weight since assignment to protocol; 2) major organ failure or medical conditions unresponsive to treatment (e.g. respiratory distress, icterus, uremia, intractable diarrhea or persistent vomiting); 3) surgical complications unresponsive to immediate intervention (e.g. uncontrolled bleeding, vascular graft/circulation failure, infection, wound dehiscence); 4) complete anorexia persisting for more than 4 days. The attending veterinarian will have final authority for the decision to euthanize animals based on their professional judgement.

For rats, subjects will be euthanized upon conclusion of their assigned experiments. The proposed methods of euthanasia also are consistent with the recommendations of the panel on euthanasia of the AVMA.

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Redacted by agreement

RESOURCE SHARING PLAN

As per data management policies at the University of Mississippi Medical Center (UMMC) and in accordance with NIH policy, all data from these studies will be stored in both hardcopy and electronic form. Sharing of data is available via electronic formats only. Electronic data are stored off-campus on servers maintained by the Division of Information Services at UMMC, who also maintain all software licenses and hardware. The servers use a Microsoft environment, with original data in raw and analyzed form stored in Microsoft Excel. Originals and backups of these data are maintained by the PI.

Dissemination of data will occur through peer-reviewed publications, in accordance with journal data management policies. In addition to standard research reports, all data are freely available upon request directly to the PI using her UMMC email address (dplatt@umc.edu). Any request will be reviewed on a case-by-case team with the investigative team for this project. The data generated from these studies will not result in proprietary information and/or predictable Intellectual Property (subject to change).

An important resource used extensively in this research is novel compounds. All experimental compounds obtained for this proposal are from Redacted by agreement and are non-development compounds that may be covered under patent but are not considered viable as potential drugs or products. All relevant chemical information for the compounds included in this proposal have been published in peer-reviewed journals and/or are available upon request. The experimental compounds are provided to UMMC consistent with policies of the University of Mississippi Medical Center and the Redacted by agreement Redacted by agreement under agreements outlined via a Material Transfer Agreement. All inquiries regarding compounds will be handled directly by Redacted by, in accordance with Redacted by agreement policies.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

The synthesis and authentication of several ligands employed in this research will occur in Redacted by laboratory at Redacted by agreement. The compounds will be analyzed first by thin layer chromatography on silica gel and alumina TLC plates using three solvent systems. After purification by medium pressure column chromatography, the ligands will be analyzed by proton NMR spectroscopy and low-resolution mass spectroscopy. Once this is completed, the compounds will be analyzed for purity by high performance liquid chromatography. Once high purity > 98-99% is shown, extensive proton NMR will be carried out including NOSEY experiments, which will then be checked rigorously by 1D NOE experiments. At this point, the C-13 spectrum of the ligands will be generated, accompanied by the NMR high resolution experiments: ROSEY, HMQC, HMBC, etc. The high resolution mass spectrum will deliver the empirical formula and the number of carbon atoms and protons will be compared carefully with the numbers in the H-NMR and C-13 spectra of the ligands to assure concordance.

Finally, a crystal structure of the target ligands will be determined by Redacted by agreement NIH-supported institute. Final purity also can be established by a CHN analysis. For *in vitro* liver microsomal studies carried out in Redacted by on HLM (Human Liver Microsomes) and MLM (Mouse Liver Microsomes), the studies routinely will be conducted in triplicate with n = 6. These *in vitro* studies will be verified in rat PK studies by Dr. Redacted by agreement using MALDI-TOF and tandem ms/ms mass spectroscopy. Metabolites will be identified by MALDI-TOF spectrometry and ms/ms, after which they will be independently verified by synthesis for comparison. Cytotoxicity studies on the HEP-G2 cells will be run in triplicate with an n=3 for each study.

Other drugs and compounds are acquired from commercial sources with noted purity certificates. Samples of these ligands are routinely sent to Redacted by laboratory for analysis on a random basis (purity measures have been remarkably consistent with manufacturer's claims over the past ~20 years).

Recipient Information

1. Recipient Name
UNIVERSITY OF MISSISSIPPI MEDICAL CENTER
2500 N STATE ST

JACKSON, MS 39216

2. Congressional District of Recipient
03

3. Payment System Identifier (ID)
1646008520A2

4. Employer Identification Number (EIN)
646008520

5. Data Universal Numbering System (DUNS)
928824473

6. Recipient's Unique Entity Identifier

7. Project Director or Principal Investigator
Donna M Platt, PHD
Associate Professor
dplatt@umc.edu
601 984 5896

8. Authorized Official
Tenay Spann
tmspann@umc.edu
601-815-3504

Federal Agency Information

9. Awarding Agency Contact Information
Celia B. Herlihy

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
celia.herlihy@nih.gov
301.443.4705

10. Program Official Contact Information
MARK EGLI
Scientific Review Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
megli@mail.nih.gov
301-594-6382

Federal Award Information

11. Award Number
5R01AA029023-02

12. Unique Federal Award Identification Number (FAIN)
R01AA029023

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol

15. Assistance Listing Number
93.273

16. Assistance Listing Program Title
Alcohol Research Programs

17. Award Action Type
Non-Competing Continuation

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 08/01/2021 – **End Date** 07/31/2022	
20. Total Amount of Federal Funds Obligated by this Action	$443,607
20 a. Direct Cost Amount	$301,991
20 b. Indirect Cost Amount	$141,616
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$443,607
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$443,607
26. Project Period Start Date 09/15/2020 – **End Date** 07/31/2025	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$914,815

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Jeffrey Thurston

30. Remarks

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

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

SECTION I – AWARD DATA – 5R01AA029023-02

Principal Investigator(s):
Donna M Platt, PHD

Award e-mailed to: ORSPpostaward@umc.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $443,607 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSISSIPPI MED CTR in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 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 recipient when funds are drawn down or otherwise requested 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 Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number R01AA029023. 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 direct questions to the Federal Agency contacts.

Sincerely yours,

Jeffrey Thurston
Grants Management Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Salaries and Wages	$127,906
Fringe Benefits	$35,303
Personnel Costs (Subtotal)	$163,209
Consultant Services	$4,050
Materials & Supplies	$13,500
Travel	$3,600
Other	$73,125
Subawards/Consortium/Contractual Costs	$44,507
Federal Direct Costs	$301,991
Federal F&A Costs	$141,616
Approved Budget	$443,607
Total Amount of Federal Funds Authorized (Federal Share)	$443,607
TOTAL FEDERAL AWARD AMOUNT	$443,607
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$443,607

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$443,607	$443,607
3	$510,067	$510,067
4	$472,924	$472,924
5	$466,274	$466,274

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

Fiscal Information:

Payment System Identifier: 1646008520A2
Document Number: RAA029023A
PMS Account Type: P (Subaccount)
Fiscal Year: 2021

IC	CAN	2021	2022	2023	2024
AA	8470415	$443,607	$510,067	$472,924	$466,274

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

NIH Administrative Data:
PCC: AN E / **OC**: 41025 / **Released**: Thurston, Jeffrey 07/19/2021
Award Processed: 07/20/2021 12:09:38 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AA029023-02

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 – STANDARD TERMS AND CONDITIONS – 5R01AA029023-02

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).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

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) R01AA029023. 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/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AA SPECIFIC AWARD CONDITIONS – 5R01AA029023-02

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This award is issued in accordance with NIH Fiscal Policies in effect for FY 2021 (see NIH Guide Notice NOT-OD-21-058).

SALARY LIMITATION: None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Current salary cap levels can be found at http://grants1.nih.gov/grants/policy/salcap_summary.htm.

INFORMATION: This award includes funds awarded for consortium activity. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS).

SPREADSHEET SUMMARY
AWARD NUMBER: 5R01AA029023-02

INSTITUTION: UNIVERSITY OF MISSISSIPPI MED CTR

Budget	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$127,906	$127,906	$127,906	$127,906
Fringe Benefits	$35,303	$35,303	$35,303	$35,303
Personnel Costs (Subtotal)	$163,209	$163,209	$163,209	$163,209
Consultant Services	$4,050	$4,050	$4,050	$4,050
Materials & Supplies	$13,500	$13,500	$13,500	$13,500
Travel	$3,600	$3,600	$3,600	$3,600
Other	$73,125	$116,002	$92,039	$87,749
Subawards/Consortium/Contractual Costs	$44,507	$44,507	$44,507	$44,507
TOTAL FEDERAL DC	$301,991	$344,868	$320,905	$316,615
TOTAL FEDERAL F&A	$141,616	$165,199	$152,019	$149,659
TOTAL COST	$443,607	$510,067	$472,924	$466,274

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	55%	55%	55%	55%
F&A Cost Base 1	$257,484	$300,361	$276,398	$272,108
F&A Costs 1	$141,616	$165,199	$152,019	$149,659

A. COVER PAGE

Project Title: GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol	
Grant Number: 5R01AA029023-02	**Project/Grant Period:** 09/15/2020 - 07/31/2025
Reporting Period: 09/15/2020 - 07/31/2021	**Requested Budget Period:** 08/01/2021 - 07/31/2022
Report Term Frequency: Annual	**Date Submitted:** 06/07/2021
Program Director/Principal Investigator Information: DONNA M PLATT , BA MA PHD **Phone Number:** 16019845896 **Email:** dplatt@umc.edu	**Recipient Organization:** UNIVERSITY OF MISSISSIPPI MED CTR UNIVERSITY OF MISSISSIPPI MEDICAL CENTER 2500 N STATE STREET JACKSON, MS 392164500 **DUNS:** 928824473 **EIN:** 1646008520A2 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: TENAY SPANN 2500 North State Street Jackson, MS 392164505 **Phone number:** 601-815-3504 **Email:** tmspann@umc.edu	**Signing Official:** JOSH CLARK 2500 North State Street Jackson, MS 39110 **Phone number:** 601-815-5000 **Email:** jtclark2@umc.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The development of pharmacological and behavioral strategies that universally reduce alcohol consumption and/or eliminate craving continues to be a challenge for researchers. Alcohol's ability to potentiate the activity of gamma-aminobutyric acid (GABA) at GABA-A receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both human subjects and laboratory animals, making this system an attractive candidate for the development of therapeutics. The complex molecular biology of GABA-A receptors raises the possibility that subtype-selective agents might be developed with therapeutic specificity against alcohol use disorders (AUDs). It is this basic premise that has guided the studies conducted in our laboratory over the past 15 years.

Our previous research has focused on GABA-A receptor subtypes that are sensitive to classic benzodiazepine-type ligands, and we have shown that alpha5GABA-A and alpha2/3GABA-A, but not alpha1GABA-A, receptors play a key role in the reinforcing and discriminative stimulus (DS) effects of alcohol in monkeys and the relapse-like effects of alcohol in rats. However, alcohol has been shown to modulate not only these "diazepam-sensitive" receptors, but also those insensitive to benzodiazepines (i.e., GABA-A receptors containing alpha4 and alpha6 subunits). GABA-A receptors containing alpha4 or alpha6 subunits are comprised of beta subunits, and either a gamma or delta subunit. Important differences exist between these populations of "diazepam-insensitive" receptors. In this regard, gamma-containing receptors (i.e., alpha4gammaGABA-A and alpha6gammaGABA-A receptors) are located synaptically and extrasynaptically and mediate both phasic and tonic inhibition of neurons, whereas delta-containing receptors (i.e., alpha4deltaGABA-A and alpha6deltaGABA-A receptors) are located extrasynaptically and contribute to tonic inhibition only. Our prior findings indicate a clear role for several extrasynaptically and synaptically located GABA-A receptors in alcohol's behavioral effects. However, the contribution of extrasynaptically located alpha4beta delta and alpha6beta delta GABA-A receptors remains controversial, with the literature supporting both a facilitative and inhibitory role for these receptors. Thus, goals of the proposed research are to resolve the role of gamma- vs. delta-containing alpha4 and alpha6GABA-A receptors in the DS, reinforcing and relapse-inducing effects of alcohol and determine whether ligands targeting these receptors exhibit therapeutic specificity against alcohol-maintained behavior.

In the clinic, pharmacotherapies typically are one of the last options to be offered to the AUD patient; rather, behavioral therapies are the approach of choice. However, patient outcomes generally are improved when behavioral therapies are combined with adjunctive pharmacotherapies. To model "medication-assisted treatment", we have developed a resurgence model of contingency management therapy. Thus, a final goal of our proposed research is to apply what we have learned previously regarding the therapeutic utility of selective GABAergic drugs alone and begin to evaluate these drugs as adjunctive pharmacotherapies to augment behavioral therapy.

Specific Aim 1 will establish the involvement of gamma-containing vs. delta-containing alpha4 and alpha6GABA-A receptors in the DS, reinforcing and relapse-inducing effects of alcohol. We will use novel ligands selective for gamma- vs. delta-containing alpha4 and alpha6GABA-A receptors and models of interoceptive effects, alcohol seeking and taking established in our laboratory with rodent and primate species. We hypothesize that delta-containing GABA-A receptors are key mediators of the reinforcing, but not DS or relapse-inducing, effects of alcohol. A novel contribution of this project will be to delineate the roles of alpha4delta, alpha6delta, alpha4gamma, and alpha6deltaGABA-A receptors, for which very little behavioral data exists.

Specific Aim 2 will establish the potential therapeutic utility of alpha4delta, alpha6delta, alpha4gamma, and alpha6gamma GABA-A ligands by determining the extent to which they alter performance maintained by a nondrug reinforcer (sucrose) and/or induce undesirable side effects using a quantitative observation procedure.

Specific Aim 3 will investigate the utility of selective GABAergic ligands with favorable side effect profiles (identified in current and past project periods) to serve as adjunctive pharmacotherapies in a model of medication-assisted treatment. These studies will make use of a novel resurgence model of contingency management developed recently in our laboratory and, initially, evaluate ligands that either mimic (e.g., alpha2/3GABA-A agonists) or attenuate (e.g., alpha5GABA-A inverse agonists) the behavioral effects of alcohol.

Integration of results from the aims will continue to yield needed information about neuropharmacological mechanisms underlying the addictive effects of alcohol and begin to identify clinical scenarios in which specific pharmacological approaches might improve patient outcomes.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : Platt B2-Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the second year of R01 AA029023, we will continue to follow the timeline, as outlined in the grant proposal. We will carry on with planned self-administration studies in both alcohol drinking and sucrose-drinking groups of monkeys. We expect to be able to establish complete dose-response functions for bretazenil, XHe-III-74, and Thio-4-PIOL. The outcomes of experiments with these compounds, together with the results from completed experiments with gaboxadol, will provide us with a general understanding of the role of alpha4/alpha6delta GABA-A receptors in the reinforcing effects of alcohol in monkeys. Further, comparisons between the alcohol- and sucrose-maintained groups will allow us to determine the specificity of any modulation by the compounds. Observation studies will occur concurrently with self-administration studies allowing us to determine behavioral profiles for the compounds in both groups of monkeys. If time allows in the second year, we will initiate studies with GHB. We will need to add GHB to our Schedule I DEA license prior to obtaining the compound and starting studies; but, we do not anticipate any difficulties accomplishing this necessary first step. We will continue to add females to the self-administration studies as they become available for purchase, or available for transfer from other researchers.

We plan to instrument and train monkeys in the alcohol discrimination procedure. Based on our experience with this procedure, we expect training to take at least 3-4 months before monkeys can reliably discriminate the training dose of alcohol from water. Once monkeys can begin testing, we will establish dose-response functions for experimental compounds, beginning with bretazenil (expected to, at least partially, reproduce the discriminative stimulus effects of alcohol). As with the self-administration studies, we will begin studies in available male monkeys and add females as we can.

All rat-related effort in the next year will be devoted to planned cue-induced reinstatement studies. Because we have been able to hire a rat-only technician and already are training a group of rats to self-administer alcohol using a sucrose-fading procedure, we expect to be able to complete studies with both gaboxadol and XHe-III-74, at least. Ideally, we also will be

able to begin studies with GHB. Note that these studies include both male and female subjects and are powered adequately to detect sex differences.

B.2 (Platt B2-Accomplishments.pdf)

In **Year 1** of **R01 AA029023-01**, we have made progress on all three of our specific aims as described below.

The overall goal of **Specific Aim 1** is to establish the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the discriminative stimulus, reinforcing and relapse-inducing effects of alcohol. Our efforts in this first year have been devoted to two primary objectives. Objective 1 was to begin determining the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the reinforcing effects of alcohol. To that end, we have trained a group of five rhesus monkeys (4 males, 1 female) to self-administer alcohol using a step-wise induction procedure and schedule-induced polydipsia technique. Once we established reliable self-administration in the group, we began evaluating the effects of experimental compounds. Recall that monkeys receive pretreatments of doses of the compounds for five consecutive days and until intake stabilizes. This allows us to determine the extent to which monkeys develop tolerance or sensitization to an observed effect. Further, we re-establish baseline self-administration between testing different doses of a compound to ensure that baseline responding/intake does not shift across the course of the study. The first compound that we have investigated in the alcohol-maintained monkeys (and determined a complete dose-response function for) is gaboxadol, a partial agonist that acts selectively at extrasynaptic α4/α6δGABA$_A$ receptors. We find that the highest dose evaluated (3 mg/kg) significantly enhances alcohol self-administration above levels observed after vehicle injection. This observation is consistent with previous findings in rats, but not mice. Currently, we are evaluating the effects of bretazenil on alcohol reinforcement. Bretazenil also is a partial agonist, but is unusual in that it binds at GABA$_A$ receptors containing α1, α2, α3, α4, α5 and α6 subunits. A previous study in which human subjects received alcohol plus either bretazenil or diazepam (only binds at α1, α2, α3, and α5 subunits) showed that bretazenil + alcohol induced a greater behavioral effect than diazepam + alcohol. We believe that this finding supports a facilitative role for α4 and/or α6GABA$_A$ receptors in the behavioral effects of alcohol. Therefore, we expect bretazenil to enhance the reinforcing effects of alcohol.

The second objective associated with **Specific Aim 1** was to begin assessing the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the relapse-inducing effects of alcohol. A necessary first step before initiating planned relapse studies was to hire a technical staff person to handle the day-to-day husbandry and experimental tasks associated with the rat studies. We have successfully hired a Master's level individual (Ms. Bailey McPhail) into a Researcher II position to serve as a rat technician. Currently, she is training male and female Wistar rats to self-administer alcohol using a standard sucrose-fade procedure. Once trained, the rats will begin planned alcohol cue-induced reinstatement studies. We anticipate initially evaluating gaboxadol. Although in a previous study gaboxadol did not induce alcohol-seeking behavior in mice, it is not certain that the same would be true for rats. Particularly given the species differences evident in gaboxadol's capacity to modulate alcohol intake, as described above.

The overall goal of **Specific Aim 2** is to establish the potential therapeutic utility of α4δ, α6δ, α4γ, and α6γGABA$_A$ ligands by determining the extent to which they alter performance maintained by a nondrug reinforcer (sucrose) and/or induce undesirable side effects as measured using a quantitative observation procedure. In this first year, to address the question of selectivity, we have successfully trained a group of monkeys (4 males) to self-administer a sucrose solution under conditions that engender intakes similar to those of the alcohol group. In these monkeys, gaboxadol across the dose range evaluated (0.1 – 3 mg/kg) failed to alter sucrose self-administration in any significant manner. This finding suggests that the enhancement of intake by gaboxadol is specific to alcohol and not due to a generalized drug-induced increase in drinking behavior. In both groups of monkeys, behavioral observations are conducted to assess the capacity of test compounds to alter species-typical behaviors or induce typical GABAergic side effects (e.g., ataxia, sedation, etc.). With gaboxadol, we have found no evidence of ataxia or sedative-like behaviors (i.e., sleep posture, deep sedation). Across doses, the only significant change in behavior is a reduction in tactile/oral manipulation of features of the cage environment (e.g., toys, mirrors, cage elements, etc.). Interestingly, this decrease in manipulative behavior does not translate into a decrease in the ability of the monkey to press a lever to gain access to the alcohol or sucrose sipper, as 3 mg/kg gaboxadol did not alter sucrose self-administration and increased alcohol self-administration. Currently, we are assessing the effects of bretazenil in the sucrose-drinking groups and obtaining observation data in both alcohol and sucrose groups.

The overall goal of **Specific Aim 3** is to investigate the utility of selective GABAergic ligands with favorable side effect profiles to serve as adjunctive pharmacotherapies in a model of medication-assisted treatment (i.e., a novel resurgence model of contingency management developed recently in our laboratory; MAT). To that end, we have spent the first year of the project period completing two resurgence studies that were presented as preliminary data in the grant application.

Our first study was an evaluation of putative cognitive enhancing drugs as adjunctive pharmacotherapies in our rat resurgence model of MAT. Rats were trained to self-administer alcohol orally under a fixed-ratio schedule (Phase 1). Once rats stably self-administered pharmacologically-relevant doses of alcohol, the alcohol was removed and the behavioral treatment initiated. Alternative reinforcers were delivered contingent on the rats withholding responses on the alcohol-paired lever (i.e., for every 10-s without a lever press, sweetened condensed milk was delivered; Phase 2). Under these conditions, alcohol seeking (as measured by lever presses to the alcohol-paired lever) decreased. When the behavioral treatment was terminated (i.e., milk delivery ceased), rats resumed alcohol seeking, despite the fact that alcohol was not available. To model a MAT approach, we administered saline or doses of the α5GABA$_A$ inverse agonist RY-023 or the glycine partial agonist d-cycloserine in conjunction with the behavioral therapy (Phase 2) and upon termination of the behavioral therapy (Phase 3). We found that rats receiving RY-023, but not d-cycloserine, in conjunction with the behavioral therapy exhibited more rapid extinction of behavior in Phase 2 and failed to exhibit the resurgence of alcohol seeking in Phase 3. These results suggest that, indeed, the addition of a pharmacotherapy to a behavioral therapy reduces the likelihood of relapse compared to the behavioral therapy alone. Further, the results suggest that cognitive enhancers targeting the GABA$_A$ receptor system, rather than the glutamatergic system, may be more effective in the context of MAT for alcohol use disorder. This study is complete and is being prepared for publication.

An objective for the first year of the project period was to shift our resurgence studies from rats to primarily monkeys. We have been successful in this endeavor. Six monkeys (all male) are trained in a resurgence procedure very similar to that described above for rats. The only substantial differences are some parameter values (e.g., FR 50 for monkeys; FR 2 for rats) and a different alternative reinforcer (i.e., flavored pellets for monkeys; sweetened condensed milk for rats). To model MAT, we have opted to study the effects of naltrexone initially, as this drug has been used in the context of MAT in humans with alcohol use disorder and should serve as a positive control for future studies. Various doses of naltrexone or vehicle were administered during Phase 2 along with the behavioral therapy, and continued into Phase 3 when the behavioral therapy was terminated. To date, five of six monkeys have completed the study, and individual differences are evident in the results. In the monkeys that have finished, naltrexone only facilitated extinction of alcohol-seeking behavior in Phase 2 in two of five monkeys. This finding differs from what we have reported previously in rats with naltrexone, but likely reflects the dominant role of the alternative reinforcer in controlling elimination of behavior in the monkeys. In future studies, we could alter the schedule under which the alternative reinforcer is delivered (i.e., make it "leaner"), if our primary interest is in manipulating Phase 2 behavior. Despite having few noticeable effects in Phase 2, naltrexone reliably reduced or eliminated resurgence in Phase 3 in four of five monkeys. These favorable results with naltrexone suggest that this new resurgence procedure in monkeys is a useful and valid model for evaluating novel adjunctive pharmacotherapies to improve outcomes of behavioral therapies.

Goals not met:

In year 1, we had planned to obtain female monkeys for alcohol self-administration studies. Unfortunately, very few female monkeys are available nation-wide for purchase. For example, at last check, the Wisconsin National Primate Research Center had no females available. The Oregon National Primate Research Center has stated that external requests for females are taking over 12-18 months to process; the same is true for the California Primate Research Center. We have submitted our request to each Center. To compensate for this lack of availability, we have been able acquire some females from our internal colony, as they rotate off of other investigators' studies. These animals typically are not naïve to operant studies, but are naïve to alcohol. We will continue to pursue the purchase of females as they become available from the National Primate Research Centers.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Golani LK, Platt DM, Rüedi-Bettschen D, Edwanker C, Huang S, Poe MM, Furtmüller R, Sieghart W, Cook JM, Rowlett JK. 8-Substituted Triazolobenzodiazepines: *In Vitro* and *In Vivo* Pharmacology in Relation to Structural Docking at the α1 Subunit-Containing $GABA_A$ Receptor. Frontiers in pharmacology. 2021;12:625233. PubMed PMID: 33959005; PubMed Central PMCID: PMC8094182; DOI: 10.3389/fphar.2021.625233.
Complete	Reeves-Darby JA, Berro LF, Rowlett JK, Platt DM. Enhancement of cue-induced reinstatement of alcohol seeking by acute total sleep restriction in male Wistar rats. Pharmacology, biochemistry, and behavior. 2021 June;205:173188. PubMed PMID: 33845082; PubMed Central PMCID: PMC8164999; DOI: 10.1016/j.pbb.2021.173188.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	Platt, Donna M	BA,MA,PHD	PD/PI	EFFORT					NA
	Y	Redacted by agreement	PHD	Co-Investigator						NA
	Y		PHD,MS,BA	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

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Redacted by agreement

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?
Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?
NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?
Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

A challenge that we are facing is the lack of female monkeys available for purchase from external sources (e.g., National Primate Research Centers; NPRCs). NPRCs report either no female monkeys available to outside investigators, or significant delays (12-18 months) in availability. We have submitted requests to all NPRCs that are accepting them, so we anticipate that we will be able to obtain females at some point in the future. To address this problem more immediately, though, we are obtaining female monkeys from our internal colony (i.e., females that are suitable for transfer from other protocols/investigators) whenever possible. These females typically have experience in operant procedures, but not with alcohol. Our strategy is to add these "internal" monkeys across procedures associated with this grant, and to then do the same with external, naïve females. In that way, females in each procedure will be balanced for previous experience. Note, that the lack of females does not hinder our ability to carry out planned studies. We have a sufficient number of males to collect data in each procedure. Rather, the lack of females hinders our ability to evaluate sex-specific hypotheses at this time.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	DUNS	Congressional District	Address
Primary: UNIVERSITY OF MISSISSIPPI MED CTR	928824473	MS-003	2500 N STATE STREET JACKSON, MS 392164505
University of Wisconsin-Milwaukee	627906399	WI-004	3210 N Cramer Street Milwaukee, WI 532113029

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

A. COVER PAGE

Project Title: GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol	
Grant Number: 5R01AA029023-03	**Project/Grant Period:** 09/15/2020 - 07/31/2025
Reporting Period: 08/01/2021 - 07/31/2022	**Requested Budget Period:** 08/01/2022 - 07/31/2023
Report Term Frequency: Annual	**Date Submitted:** 06/13/2022
Program Director/Principal Investigator Information: DONNA M PLATT , BA MA PHD **Phone Number:** 16019845896 **Email:** dplatt@umc.edu	**Recipient Organization:** UNIVERSITY OF MISSISSIPPI MED CTR UNIVERSITY OF MISSISSIPPI MEDICAL CENTER 2500 N STATE STREET JACKSON, MS 392164500 **DUNS:** 928824473 **EIN:** 1646008520A2 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: FELICIA CLERK 2500 North State Street Jackson, MS 392164505 **Phone number:** 601-815-5004 **Email:** fclerk@umc.edu	**Signing Official:** ELIZABETH D BELL UNIVERSITY OF MISSISSIPPI MEDICAL CENTER 2500 NORTH STATE STREET JACKSON, MS 392164505 **Phone number:** 601-815-5052 **Email:** EBELL@UMC.EDU
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The development of pharmacological and behavioral strategies that universally reduce alcohol consumption and/or eliminate craving continues to be a challenge for researchers. Alcohol's ability to potentiate the activity of gamma-aminobutyric acid (GABA) at GABA-A receptors has been implicated as a key mechanism underlying the abuse-related effects of alcohol in both human subjects and laboratory animals, making this system an attractive candidate for the development of therapeutics. The complex molecular biology of GABA-A receptors raises the possibility that subtype-selective agents might be developed with therapeutic specificity against alcohol use disorders (AUDs). It is this basic premise that has guided the studies conducted in our laboratory over the past 15 years.

Our previous research has focused on GABA-A receptor subtypes that are sensitive to classic benzodiazepine-type ligands, and we have shown that alpha5GABA-A and alpha2/3GABA-A, but not alpha1GABA-A, receptors play a key role in the reinforcing and discriminative stimulus (DS) effects of alcohol in monkeys and the relapse-like effects of alcohol in rats. However, alcohol has been shown to modulate not only these "diazepam-sensitive" receptors, but also those insensitive to benzodiazepines (i.e., GABA-A receptors containing alpha4 and alpha6 subunits). GABA-A receptors containing alpha4 or alpha6 subunits are comprised of beta subunits, and either a gamma or delta subunit. Important differences exist between these populations of "diazepam-insensitive" receptors. In this regard, gamma-containing receptors (i.e., alpha4gammaGABA-A and alpha6gammaGABA-A receptors) are located synaptically and extrasynaptically and mediate both phasic and tonic inhibition of neurons, whereas delta-containing receptors (i.e., alpha4deltaGABA-A and alpha6deltaGABA-A receptors) are located extrasynaptically and contribute to tonic inhibition only. Our prior findings indicate a clear role for several extrasynaptically and synaptically located GABA-A receptors in alcohol's behavioral effects. However, the contribution of extrasynaptically located alpha4beta delta and alpha6beta delta GABA-A receptors remains controversial, with the literature supporting both a facilitative and inhibitory role for these receptors. Thus, goals of the proposed research are to resolve the role of gamma- vs. delta-containing alpha4 and alpha6GABA-A receptors in the DS, reinforcing and relapse-inducing effects of alcohol and determine whether ligands targeting these receptors exhibit therapeutic specificity against alcohol-maintained behavior.

In the clinic, pharmacotherapies typically are one of the last options to be offered to the AUD patient; rather, behavioral therapies are the approach of choice. However, patient outcomes generally are improved when behavioral therapies are combined with adjunctive pharmacotherapies. To model "medication-assisted treatment", we have developed a resurgence model of contingency management therapy. Thus, a final goal of our proposed research is to apply what we have learned previously regarding the therapeutic utility of selective GABAergic drugs alone and begin to evaluate these drugs as adjunctive pharmacotherapies to augment behavioral therapy.

Specific Aim 1 will establish the involvement of gamma-containing vs. delta-containing alpha4 and alpha6GABA-A receptors in the DS, reinforcing and relapse-inducing effects of alcohol. We will use novel ligands selective for gamma- vs. delta-containing alpha4 and alpha6GABA-A receptors and models of interoceptive effects, alcohol seeking and taking established in our laboratory with rodent and primate species. We hypothesize that delta-containing GABA-A receptors are key mediators of the reinforcing, but not DS or relapse-inducing, effects of alcohol. A novel contribution of this project will be to delineate the roles of alpha4delta, alpha6delta, alpha4gamma, and alpha6deltaGABA-A receptors, for which very little behavioral data exists.

Specific Aim 2 will establish the potential therapeutic utility of alpha4delta, alpha6delta, alpha4gamma, and alpha6gamma GABA-A ligands by determining the extent to which they alter performance maintained by a nondrug reinforcer (sucrose) and/or induce undesirable side effects using a quantitative observation procedure.

Specific Aim 3 will investigate the utility of selective GABAergic ligands with favorable side effect profiles (identified in current and past project periods) to serve as adjunctive pharmacotherapies in a model of medication-assisted treatment. These studies will make use of a novel resurgence model of contingency management developed recently in our laboratory and, initially, evaluate ligands that either mimic (e.g., alpha2/3GABA-A agonists) or attenuate (e.g., alpha5GABA-A inverse agonists) the behavioral effects of alcohol.

Integration of results from the aims will continue to yield needed information about neuropharmacological mechanisms underlying the addictive effects of alcohol and begin to identify clinical scenarios in which specific pharmacological approaches might improve patient outcomes.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the third year of R01 AA029023, we will continue to follow the timeline, as outlined in the grant proposal. We will carry on with planned self-administration studies in both alcohol drinking and sucrose-drinking groups of monkeys. We expect to be able to finish dose-response functions for Cook 6 and DK-I-56-I. We also expect to be able to complete a dose-response function with GHB, an agonist at alpha4delta GABA-A receptors. Of note, GHB is designated as a DEA Schedule 1 compound. However, we already have undertaken the necessary steps to extend our DEA license to include Schedule 1 compounds. We are in the process of putting together a request to the NIDA Drug Synthesis program for a sufficient quantity of GHB for our proposed studies. The outcomes of experiments with these compounds, together with the results from completed experiments from Project Periods 1 and 2, will provide us with a general understanding of the role of delta-containing vs. gamma-containing alpha4 and alpha6 GABA-A receptors in the reinforcing effects of alcohol in monkeys. Further, comparisons between the alcohol- and sucrose-maintained groups will allow us to determine the specificity of any modulation by the compounds. Observation studies will occur concurrently with self-administration studies allowing us to determine behavioral profiles for the compounds in both groups of monkeys. Finally, we will continue to add females to the self-administration studies as they become available for purchase, or available for transfer from other researchers.

Most rat-related effort in the next project period will continue to be devoted to planned cue-induced reinstatement studies (but see below). We expect to be able to complete studies with gaboxadol and XHe-III-74 and, ideally, begin studies with GHB and/or DK-I-56-I. We have just purchased additional male and female rats to supplement the current subject population. A near-term objective will be to have these animals trained to self-administer alcohol as soon as they are acclimated to the lab.

Our resurgence studies in monkeys will continue in year 3 with a focus on drugs (inverse agonists and agonists) targeting the alpha5GABA-A receptor. As monkeys complete the planned Aim 1 self-administration studies, they will move to planned Aim 3

resurgence studies in order to increase the "N".

We request a modification to our original plans for alcohol discrimination studies. We propose to conduct initial discrimination studies in rats implanted with intragastric catheters and trained to discriminate alcohol (2 g/kg, i.g.) from water. Any positive effects in the rat studies then would be followed up in monkeys trained to discriminate alcohol (2 g/kg, i.g.) from water, as originally proposed. This decision is driven by two factors: 1) the prevailing evidence suggests that the majority of compounds to be tested will neither mimic nor modulate the discriminative stimulus effects of alcohol (an important, but negative finding), and 2) the relative scarcity of monkeys (especially females). This modified approach potentially will allow for a more efficient use of available monkeys, while still addressing the outstanding question of the role of delta-containing vs. gamma-containing alpha4 and alpha6 GABA-A receptors in the interoceptive effects of alcohol.

B.2 (B2-Accomplishments.pdf)

In **Year 2** of **R01 AA029023-02**, we continue to make progress on all three of our specific aims as described below.

The overall goal of **Specific Aim 1** is to establish the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the discriminative stimulus, reinforcing and relapse-inducing effects of alcohol. Our efforts in this second year remain devoted to two primary objectives. Objective 1 was to begin determining the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the reinforcing effects of alcohol. We have made substantial progress towards this objective. We have completed studies with bretazenil, a partial agonist that binds at GABA$_A$ receptors containing α1, α2, α3, α4, α5 and α6 subunits. Our data show that moderate doses of the drug enhance alcohol drinking by ~170% compared to intake after vehicle. That bretazenil enhanced alcohol intake agrees with a previous study in which human subjects received alcohol plus either bretazenil or diazepam (which only modulates α1, α2, α3, and α5 subunit-containing GABA$_A$ receptors) and showed that bretazenil + alcohol induced a greater behavioral effect than diazepam + alcohol. We believe that this finding supports a facilitative role for α4 and/or α6GABA$_A$ receptors in the behavioral effects of alcohol.

In the first project period, we had completed a dose response function for gaboxadol, a partial agonist that acts selectively at extrasynaptic α4/α6δGABA$_A$ receptors. We found that the highest dose evaluated (3 mg/kg) significantly enhanced alcohol self-administration above levels observed after vehicle injection. This observation is consistent with previous findings in rats, but not mice. It also suggests that the δ subunit may be key to the enhancement that we observe. This latter notion is supported by our findings with XHe-III-74, an agonist that acts selectively at synaptic/extrasynaptic α4/α6γGABA$_A$ receptors. Although XHe-III-74 increased alcohol drinking, the level was not sufficiently above vehicle levels to be considered significant. We currently are obtaining full dose-response functions for Cook 6, a novel compound with agonist activity at α6γGABA$_A$ receptors, and DK-I-56-I, a novel compound with agonist activity at α6δ/γGABA$_A$ receptors. Based on the hypothesis that modulation of δ subunits facilitates alcohol drinking, we do not expect Cook 6 to alter drinking behavior. It is more difficult to predict if or how DK-I-56-I will alter drinking. It may be that we will see some enhancement that can be attributed to modulation of δ and/or α6 subunits. At this point, we have not observed marked sex differences in outcomes, but note that our number of females remains low (see below for more discussion).

The second objective associated with **Specific Aim 1** was to begin assessing the involvement of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the relapse-inducing effects of alcohol. Since year 1, we have been able to train male and female Wistar rats (n=10/sex) to self-administer alcohol using a standard sucrose-fade procedure and have initiated planned alcohol cue-induced reinstatement studies. Note that we have made the choice to use a within-subjects experimental design to reduce the number of animals required for these studies. This is a somewhat unusual approach for reinstatement studies in rats and raises the question of whether rats will continue to respond to the alcohol-paired cue with repeated exposure over a long period-of-time. For this reason, we have modified the reinstatement protocol to always pair a drug test with a vehicle test, and to always return to alcohol self-administration and extinction between every test (e.g., Self admin → extinguish → paired vehicle 1; Self admin → extinguish → drug dose 1; Self admin → extinguish → paired vehicle 2; Self admin → extinguish → drug dose 2). This modification significantly lengthens the amount of time needed to complete a full dose-response function. Nevertheless, we believe it is necessary to address head-on the anticipated critiques regarding the persistence of the reinstatement effect. To date, most animals have completed a full dose-response function with bretazenil and have moved onto testing with either gaboxadol or XHe-III-74. What we can conclude thus far is: 1) alcohol cue-induced reinstatement is both robust and stable across time (i.e., the alcohol-paired cue has elicited stable alcohol seeking across 9 paired vehicle tests, which equates to 18 cycles of the self-administration-extinction-reinstatement protocol), and 2) bretazenil appears to enhance cue-induced alcohol seeking in female but not male rats. Conclusions regarding the role of γ-containing vs. δ-containing α4 and α6GABA$_A$ receptors in the relapse-inducing effects of alcohol will become clearer as we continue to test the selective drugs in this model.

The overall goal of **Specific Aim 2** is to establish the potential therapeutic utility of α4δ, α6δ, α4γ, and α6γGABA$_A$ ligands by determining the extent to which they alter performance maintained by a nondrug reinforcer (sucrose)

and/or induce undesirable side effects as measured using a quantitative observation procedure. In this second year, to address the question of selectivity, we have evaluated full dose-response functions for bretazenil and XHe-III-74 and currently are evaluating Cook 6 and DK-I-56-I in a group of monkeys trained to self-administer a sucrose solution under conditions that engender intakes similar to those of the alcohol group. Recall from Project Period 1 that, in these monkeys, gaboxadol across the dose range evaluated failed to alter sucrose self-administration in any significant manner. Likewise, bretazenil did not induce significant changes in sucrose drinking. XHe-III-74 did not modulate sucrose drinking, but it also did not modulate alcohol drinking. These findings suggest that the enhancement of intake by bretazenil and gaboxadol is specific to alcohol and not due to a generalized drug-induced increase in drinking behavior. In both groups of monkeys, behavioral observations are conducted to assess the capacity of test compounds to alter species-typical behaviors or induce typical GABAergic side effects (e.g., ataxia, sedation, etc.). As reported in year 1, gaboxadol does not appear to induce ataxia or sedative-like behaviors (i.e., sleep posture, deep sedation). Across doses, the only significant change in behavior was a reduction in tactile/oral manipulation of features of the cage environment (e.g., toys, mirrors, cage elements, etc.). Interestingly, this decrease in manipulative behavior did not translate into a decrease in the ability of the monkey to press a lever to gain access to the alcohol or sucrose sipper, as 3 mg/kg gaboxadol did not alter sucrose self-administration and increased alcohol self-administration. XHe-III-74 did not influence either alcohol or sucrose drinking, raising the possibilities that we were testing ineffective doses or that the compound was behaviorally inert. Fortunately, XHe-III-74 did induce changes in observable behavior (increased tactile/oral exploration, increased passive visual, elicited nose rubbing which has been interpreted to reflect gastrointestinal distress) indicating that we did test active doses. Observation data for bretazenil are being analyzed currently.

The overall goal of **Specific Aim 3** is to investigate the utility of selective GABAergic ligands with favorable side effect profiles to serve as adjunctive pharmacotherapies in a model of medication-assisted treatment (i.e., a novel resurgence model of contingency management developed recently in our laboratory; MAT). As planned for year 2, we have written up the results from a rat study evaluating putative cognitive enhancing drugs as adjunctive pharmacotherapies in our resurgence model of MAT that was completed in year 1. This paper currently is under review at *Neuropharmacology*.

In this past year, we also completed our first resurgence study in monkeys. The goal of this initial study was to translate the rat alcohol resurgence model to monkeys and then to evaluate naltrexone as an adjunctive pharmacotherapy in the model. We trained monkeys to orally self-administer alcohol under a fixed-ratio schedule in which every 50 lever presses resulted in access to a 4% w/v alcohol solution (Phase 1). Once self-administration was stable, alcohol was omitted and the behavioral treatment was initiated. The behavioral treatment consisted of the delivery of an alternative reinforcer contingent on the monkey withholding responses on the alcohol-paired lever (i.e., for every 60-s without a lever press, a flavored food pellet was delivered; Phase 2). Under these conditions, alcohol seeking (i.e., lever presses on the alcohol-paired lever) decreased in all monkeys. When the behavioral treatment was terminated (i.e., food pellet delivery ceased), all monkeys exhibited resurgence of alcohol seeking, increasing their responding on the alcohol-paired lever, despite lever presses having no programmed consequences (Phase 3). To model a MAT approach, naltrexone was administered in conjunction with the behavioral therapy (Phase 2) and upon termination of the behavioral therapy (Phase 3). This tapering approach to treatment (i.e., Phase 2: behavioral therapy + pharmacotherapy; Phase 3: pharmacotherapy only) was found to be the most effective arrangement of therapies in our previous study in rats. In monkeys, we found that naltrexone both facilitated extinction (Phase 2) and reduced resurgence of alcohol seeking (Phase 3) in a majority of subjects. However, the effects were not always dose-dependent or to similar degrees. These results suggest that an appropriate arrangement of behavioral and pharmacotherapies can effectively reduce relapse in a majority of subjects. Further, the favorable results with naltrexone suggest that this new resurgence procedure in monkeys is a useful and valid model for evaluating novel adjunctive pharmacotherapies to improve outcomes of behavioral therapies. The results from this study will be reported at the upcoming Research Society on Alcoholism meeting in Orlando, FL. Moving forward and as monkeys become available from Aim 1 studies, we will begin to evaluate drugs (inverse agonists and agonists) targeting the $\alpha 5GABA_A$ receptor in this model.

B.2 (B2-Accomplishments.pdf)

In year 1, we were unable to obtain the full cohort of female monkeys as planned due to shortages in supply. We have, however, been able acquire some females from our internal colony, as they rotate off of other investigators' studies. These animals typically are not naïve to operant studies, but are naïve to alcohol. To address the continuing shortages, all of the nonhuman primate researchers at UMMC have formed a standing committee with the charge of making more efficient use of our internal monkey population (over 100 animals). We anticipate being able to fulfill our need for females through this committee. The only expected consequence from this approach is that the female data will emerge later in the grant period than those from the males.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Chandler CM, Reeves-Darby J, Jones SA, Li G, Rahman MT, Cook JM, Platt DM. Modulation of relapse-like drinking in male Sprague-Dawley rats by ligands targeting the α5GABA$_A$ receptor. Neuropharmacology. 2021 November 1;199:108785. PubMed PMID: 34509495; PubMed Central PMCID: PMC8511242; DOI: 10.1016/j.neuropharm.2021.108785.
Complete	Duke AN, Tiruveedhula VVNPB, Sharmin D, Knutson DE, Cook JM, Platt DM, Rowlett JK. Tolerance and dependence following chronic alprazolam treatment in rhesus monkeys: Role of GABA$_A$ receptor subtypes. Drug and alcohol dependence. 2021 November 1;228:108985. PubMed PMID: 34500240; PubMed Central PMCID: PMC8595788; DOI: 10.1016/j.drugalcdep.2021.108985.
Complete	Berro LF, Pareek T, Reeves-Darby JA, Andersen ML, Howell LL, Platt DM, Rowlett JK. Influence of Pair-housing on Sleep Parameters Evaluated with Actigraphy in Female Rhesus Monkeys. Journal of the American Association for Laboratory Animal Science : JAALAS. 2022 March 1;61(2):165-172. PubMed PMID: 35012705; PubMed Central PMCID: PMC8956211; DOI: 10.30802/AALAS-JAALAS-21-000027.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	Platt, Donna M	BA,MA,PHD	PD/PI	EFFORT					NA
	Y	Redacted by agreement	PHD,MS,BA	Co-Investigator						NA
	Y		PHD	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?
Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?
NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?
Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

File uploaded: F3 - changes to VAS.pdf

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

F.3.b (F3 - changes to VAS.pdf)

In **Year 3** of **R01 AA029023**, we request an addition to our original plans for alcohol discrimination studies. We propose to conduct initial discrimination studies in rats implanted with intragastric catheters and trained to discriminate alcohol (2 g/kg, i.g.) from water. Because this is an addition to the project, we include below a Vertebrate Animals Section pertinent only to this new procedure. Of note, in the context of other projects, we already have an approved protocol to implant intragastric catheters in rats.

VERTEBRATE ANIMALS

Rats will be housed in the vivarium of the Redacted by agreement

1. Description of procedures. Our proposed research will investigate the role of specific GABAergic mechanisms in the discriminative stimululs effects of alcohol in rats trained to discriminate intragastrically-administered alcohol from vehicle. This study will provide needed information about specific $GABA_A$ mechanisms that may contribute to the addictive effects of alcohol and will aid identification of GABAergic ligands for the pharmacological management of alcohol abuse and relapse.

A group of rats (8 males/8 females) will serve as subjects. The rats will be of the Wistar strain and obtained from Charles River, Inc. Rats will be pair-housed in standard shoebox cages in a vivarium with regulated temperature, humidity, air exchange, and light-dark cycle (Note: rats will be housed under a reverse light-dark cycle and discrimination studies will be conducted during the dark portion of the cycle). The cages/bedding will be changed twice weekly. Supervised technicians will be responsible for daily care and feeding of all rats. Rats will have ad lib access to water and adjusted amounts of standard rat chow to maintain experimental performance. Rats will be weighed once/week, at a minimum.

2. Justifications.

Alcohol use disorders are clearly significant problems in our society. A critical step in the effective management of alcohol addiction is to establish definitive data regarding factors controlling the abuse-related effects of alcohol, and to use this information to facilitate development of effective therapeutic strategies. Rats are ideally suited for preclinical research on these questions. This species has been used in behavioral pharmacology research for over 50 years and has provided valid and reliable models of different aspects of alcohol addiction in humans. Because of the extensive use of rats (of many strains) in alcohol research, there is a large body of scientific information which will provide indispensable comparative information for proper interpretation of our research and for its application to the development of medications to treat alcohol abuse and dependence. The proposed work cannot be conducted in humans because of the addictive and/or experimental nature of the drugs under study. The research also cannot be conducted using tissue samples or biological material because a primary goal of the research is to determine how drugs alter and control behavior in models predictive of effects in humans. At this point in time, computer modeling does not provide meaningful information for research of this type, since modeling depends largely on *a priori* information that is not available without first conducting the types of studies described in this application.

The number of rats to use in behavioral experiments is a decision that involves a trade-off between using large numbers of valuable animals and assuring the reliability of data collected using smaller numbers of animals. The rat discrimination experiments proposed are designed to increase the reliability of data from small numbers by using, whenever possible, a within-subjects experimental design. This design, in which each animal serves as its own control, permits scientifically meaningful results to be obtained with fewer animals than would be required with other types of designs (e.g., an exclusively between-subjects approach). The number of rats to be used is based on typical group sizes as reported in the literature (cf. Green-Jordan & Grant, 2000).

3. Minimization of pain and distress.

The research will be conducted with veterinary supervision from the University of Mississippi Medical Center (UMMC) Center for Comparative Research (CCR). The CCR is attended by a full-time veterinary and support staff, including two full-time veterinarians, four certified veterinary technicians, and a veterinary assistant. This staff is experienced in providing high quality care to laboratory animals. Rats will be housed in Redacted by agreement facility in rooms custom designed for this research. CCR and veterinary staff provide daily husbandry and veterinary care for animals that is consistent with the Guide for Care and Use of Laboratory Animals. Redacted by

Redacted the attending veterinarian, is the Director of CCR and is Board Certified by the American College of Laboratory Animal Medicine. All animal care and experimental procedures are conducted in accordance with laws, regulations, and guidelines of the PHS Policy, Office of Laboratory Animal Welfare, United States Department of Agriculture, UMMC's Institutional Animal Care and Use Committee, and CCR institutional policies.

The CCR at UMMC has in place an active Environmental Enrichment Program to promote the humane care and psychological well-being of all experimental animals. All animals are housed in groups unless experimental design specifically requires individual housing in which case special exemption must be sought and approved by the IACUC.

The PI, laboratory personnel and animal technicians involved in the proposed research will have had instruction or demonstrated their competence in the care, use and handling of laboratory animals. We give assurance that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals. Discomfort will be limited to what is unavoidable in conducting the research. Clinical veterinarians in the CCR will be responsible for diagnosing and treating animals displaying signs of pain and distress and for medical evaluations as required.

Discomfort, distress, or pain may be associated with the intragastric catheter implantation for alcohol discrimination studies. To minimize these effects, surgeries will be conducted using strict aseptic techniques and anesthesia (inhaled isoflurane), on a warm surface. Carprofen (5 mg/kg, s.c.) will be administered pre- and post-operatively for pain, and an antibiotic (Gentamicin or Naxcel, 4-5 mg/kg) may be administered to prevent infection. Following surgery, research technicians will monitor rats continuously until they are awake. Once awake, monkeys will be monitored every 15-30 min by personnel until they take and eat treats or fruit. Veterinary and research staff will continue to monitor animals on a daily basis post-surgery for a minimum of one week. Experimental sessions will not begin until animals are fully recovered from surgery, a minimum of 5 days.

In alcohol discrimination studies, rats receive food reinforcement. Stable daily performance is maintained by restricting access to food in the animal's living quarters. During initial training, body weights are reduced to approximately 85% of ad libitum values. Once subjects respond reliably under the schedule of food reinforcement, home-cage feeding is increased to the maximum allotment that can be given without resulting in degraded performances during experimental sessions.

Rats will be habituated to their cages and housing room prior to the onset of the studies. They will be handled routinely and habituated to the injection process in order to reduce the occurrence of stress.

Across all procedures, doses of test compounds used in our proposed studies are carefully selected to provide scientifically meaningful data and are not expected to induce significant toxicity or compromise the health of the subjects. All drugs and vehicle will be pharmaceutical grade, received directly from NIH/NIDA, or received after extensive purity analysis by the laboratory of our collaborator Redacted by agreement Redacted by agreement The primary effects of the compounds are expected to be temporary and reversible. Should untoward effects occur for any reason, however, experiments will be discontinued until the cause of the problem can be identified and resolved to minimize pain and distress.

4. Euthanasia.

For rats, subjects will be euthanized upon conclusion of their assigned experiments. The proposed methods of euthanasia also are consistent with the recommendations of the panel on euthanasia of the AVMA.

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	DUNS	Congressional District	Address
Primary: UNIVERSITY OF MISSISSIPPI MED CTR	928824473	MS-003	2500 N STATE STREET JACKSON, MS 392164505
University of Wisconsin-Milwaukee	627906399	WI-004	3210 N Cramer Street Milwaukee, WI 532113029

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Recipient Information

1. Recipient Name
UNIVERSITY OF MISSISSIPPI MEDICAL CENTER
2500 N STATE ST

JACKSON, 39216

2. Congressional District of Recipient
03

3. Payment System Identifier (ID)
1646008520A2

4. Employer Identification Number (EIN)
646008520

5. Data Universal Numbering System (DUNS)
928824473

6. Recipient's Unique Entity Identifier
X59NJBFL8BJ3

7. Project Director or Principal Investigator
Donna M Platt, PHD
Associate Professor
dplatt@umc.edu
601-984-5896

8. Authorized Official
Felicia Clerk
fclerk@umc.edu
601-815-5004

Federal Agency Information

9. Awarding Agency Contact Information
Celia B. Herlihy

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
celia.herlihy@nih.gov
301.443.4705

10. Program Official Contact Information
MARK EGLI
Scientific Review Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
megli@mail.nih.gov
301-594-6382

Federal Award Information

11. Award Number
5R01AA029023-03

12. Unique Federal Award Identification Number (FAIN)
R01AA029023

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
GABA-A receptor subtype mechanisms and the abuse-related effects of alcohol

15. Assistance Listing Number
93.273

16. Assistance Listing Program Title
Alcohol Research Programs

17. Award Action Type
Non-Competing Continuation

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 08/01/2022 – **End Date** 07/31/2023	
20. Total Amount of Federal Funds Obligated by this Action	$510,067
20 a. Direct Cost Amount	$344,868
20 b. Indirect Cost Amount	$165,199
21. Authorized Carryover	
22. Offset	
23. Total Amount of Federal Funds Obligated this budget period	$510,067
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$510,067
26. Project Period Start Date 09/15/2020 – **End Date** 07/31/2025	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$1,424,882

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Jeffrey Thurston

30. Remarks

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

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

SECTION I – AWARD DATA – 5R01AA029023-03

Principal Investigator(s):
Donna M Platt, PHD

Award e-mailed to: ORSPpostaward@umc.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $510,067 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSISSIPPI MED CTR in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 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 recipient when funds are drawn down or otherwise requested 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 Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number R01AA029023. 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 direct questions to the Federal Agency contacts.

Sincerely yours,

Jeffrey Thurston
Grants Management Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Salaries and Wages	$127,906
Fringe Benefits	$35,303
Personnel Costs (Subtotal)	$163,209
Consultant Services	$4,050
Materials & Supplies	$13,500
Travel	$3,600
Other	$116,002
Subawards/Consortium/Contractual Costs	$44,507
Federal Direct Costs	$344,868
Federal F&A Costs	$165,199
Approved Budget	$510,067
Total Amount of Federal Funds Authorized (Federal Share)	$510,067
TOTAL FEDERAL AWARD AMOUNT	$510,067
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$510,067

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$510,067	$510,067
4	$472,924	$472,924
5	$466,274	$466,274

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

Fiscal Information:

Payment System Identifier: 1646008520A2
Document Number: RAA029023A
PMS Account Type: P (Subaccount)
Fiscal Year: 2022

IC	CAN	2022	2023	2024
AA	8470415	$510,067	$472,924	$466,274

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

NIH Administrative Data:
PCC: AN E / **OC**: 41025 / **Released**: Thurston, Jeffrey 07/14/2022
Award Processed: 07/15/2022 12:08:55 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AA029023-03

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

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SECTION III – STANDARD TERMS AND CONDITIONS – 5R01AA029023-03

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).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to obtain a unique entity identifier (UEI) and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a UEI 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) R01AA029023. 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/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AA SPECIFIC AWARD CONDITIONS – 5R01AA029023-03

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This award is issued in accordance with NIH FY22 Fiscal Policies (see NIH Guide Notice NOT-OD-22-105â€¯atâ€¯https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-105.html), including salary limitations as indicated below.

SALARY LIMITATION:â€¯ None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Current salary cap levels can be found at https://grants.nih.gov/grants/policy/salcap_summary.htm.

INFORMATION:â€¯ This award includes funds awarded for consortium activity.â€¯ Consortia are to be established and administered as described in the NIH Grants Policy Statement (see http://grants.nih.gov/grants/policy/policy.htm#gps).

SPREADSHEET SUMMARY
AWARD NUMBER: 5R01AA029023-03

INSTITUTION: UNIVERSITY OF MISSISSIPPI MED CTR

Budget	Year 3	Year 4	Year 5
Salaries and Wages	$127,906	$127,906	$127,906
Fringe Benefits	$35,303	$35,303	$35,303
Personnel Costs (Subtotal)	$163,209	$163,209	$163,209
Consultant Services	$4,050	$4,050	$4,050
Materials & Supplies	$13,500	$13,500	$13,500
Travel	$3,600	$3,600	$3,600
Other	$116,002	$92,039	$87,749
Subawards/Consortium/Contractual Costs	$44,507	$44,507	$44,507
TOTAL FEDERAL DC	$344,868	$320,905	$316,615
TOTAL FEDERAL F&A	$165,199	$152,019	$149,659
TOTAL COST	$510,067	$472,924	$466,274

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	55%	55%	55%
F&A Cost Base 1	$300,361	$276,398	$272,108
F&A Costs 1	$165,199	$152,019	$149,659