Notice of Award

COMPREHENSIVE CENTERS
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 12/20/2019

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Grant Number: 2P60AA009803-27
FAIN: P60AA009803

Principal Investigator(s):
PATRICIA E. MOLINA, MD

Project Title: LSUHSC-NO Comprehensive Alcohol-HIV/AIDS Research Center

Ann Clesi
COORDINATOR, OFFICE OF RESEARCH SERVICES
433 BOLIVAR ST
8th FLOOR
NEW ORLEANS, LA 701127021

Award e-mailed to: spon_proj@lsuhsc.edu

Period Of Performance:
Budget Period: 01/01/2020 – 11/30/2020
Project Period: 12/01/1996 – 11/30/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,517,259 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to LSU HEALTH SCIENCES CENTER 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 P60AA009803. 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 – 2P60AA009803-27

Award Calculation (U.S. Dollars)

Salaries and Wages	$558,333
Fringe Benefits	$224,938
Personnel Costs (Subtotal)	$783,271
Consultant Services	$4,500
Materials & Supplies	$135,249
Patient Care	$84,106
Other	$5,400
Subawards/Consortium/Contractual Costs	$54,027
Federal Direct Costs	$1,066,553
Federal F&A Costs	$450,706
Approved Budget	$1,517,259
Total Amount of Federal Funds Obligated (Federal Share)	$1,517,259
TOTAL FEDERAL AWARD AMOUNT	$1,517,259
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,517,259

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
27	$1,517,259	$1,517,259
28	$1,523,086	$1,523,086
29	$1,465,817	$1,465,817
30	$1,430,980	$1,430,980
31	$1,391,700	$1,391,700

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:	1726087770A2
Document Number:	PAA009803F
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023	2024
AA	8470437	$1,517,259	$1,523,086	$1,465,817	$1,430,980	$1,391,700

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

NIH Administrative Data:
PCC: AMAL / **OC**: 41032 / **Released**: [redacted] 12/16/2019
Award Processed: 12/20/2019 12:01:24 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2P60AA009803-27

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 – 2P60AA009803-27

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).

Carry over of an unobligated balance into the next budget period requires 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) P60AA009803. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

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

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.

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.

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

HUMAN SUBJECTS INFORMATION: The research supported by this award involves a population of **human subjects identified as "vulnerable".** Investigators who conduct research involving vulnerable populations, including pregnant women, human fetuses and neonates, prisoners, or children must follow the provisions of the regulations in Subparts B, C, and D of 45 CFR Part 46, respectively, which describe the additional protections required for these populations (https://grants.nih.gov/policy/humansubjects/policies-and-regulations/vulnerable-populations.htm).The research supported by this award involves a population of **human subjects identified as "vulnerable".** Investigators who conduct research involving vulnerable populations, including pregnant women, human fetuses and neonates, prisoners, or children must follow the provisions of the regulations in Subparts B, C, and D of 45 CFR Part 46, respectively, which describe the additional protections required for these populations (https://grants.nih.gov/policy/humansubjects/policies-and-regulations/vulnerable-populations.htm).

NIAAA DATA ARCHIVE (NIAAADA) DATA SHARING PLAN: This award is subject to the data sharing guidance outlined in NOT-AA-19-02010 (https://grants.nih.gov/grants/guide/notice-files/NOT-AA-19-020.html). The Recipient agrees to adhere to the NIAAADA Data Sharing Plan (DSP) as approved by the NIAAA Program Officer assigned to this award. Dissemination of study data will be in accord with the Recipient's approved DSP. Please note that a statement of progress on the DSP must be included in the Research Performance Progress Report (RPPR; http://grants.nih.gov/grants/rppr/index.htm) under section C.5 "Other Products and Resource Sharing". Failure to adhere to the DSP as mutually agreed upon by the Recipient and the NIAAA may result in Enforcement Actions as described in the NIH Grants Policy Statement (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_8/8.5_special_award_conditions_and_enforcement_actions.htm)
Complete NIAAADA Data Sharing Terms and Conditions can be found at https://nda.nih.gov/contribute_data_sharing_regimen.html.

STAFF CONTACTS

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

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

Program Official: Li Lin
Email: linli@mail.nih.gov **Phone**: 301-827-7749

SPREADSHEET SUMMARY
GRANT NUMBER: 2P60AA009803-27

INSTITUTION: LSU HEALTH SCIENCES CENTER

Budget	Year 27	Year 28	Year 29	Year 30	Year 31
Salaries and Wages	$558,333	$558,333	$558,333	$558,333	$558,333
Fringe Benefits	$224,938	$224,938	$224,938	$224,938	$224,938
Personnel Costs (Subtotal)	$783,271	$783,271	$783,271	$783,271	$783,271
Consultant Services	$4,500	$4,635	$4,775	$4,918	$5,065
Materials & Supplies	$135,249	$135,278	$94,784	$73,563	$45,025
Patient Care	$84,106	$84,106	$84,106	$84,106	$84,106
Other	$5,400	$2,802	$2,830	$984	$1,013
Subawards/Consortium/Contractual Costs	$54,027	$60,425	$61,973	$63,566	$65,207
TOTAL FEDERAL DC	$1,066,553	$1,070,517	$1,031,739	$1,010,408	$983,687
TOTAL FEDERAL F&A	$450,706	$452,569	$434,078	$420,572	$408,013
TOTAL COST	$1,517,259	$1,523,086	$1,465,817	$1,430,980	$1,391,700

Facilities and Administrative Costs	Year 27	Year 28	Year 29	Year 30	Year 31
F&A Cost Rate 1	47%	47%	47%	47%	47%
F&A Cost Base 1	$958,949	$962,913	$923,570	$894,834	$868,113
F&A Costs 1	$450,706	$452,569	$434,078	$420,572	$408,013

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 7826278140000

Legal Name*: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO
Department: Office of Research Services
Division: Chancellor's Office
Street1*:
Street2:
City*: NEW ORLEANS
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701127021

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Ann		Clesi	

Position/Title: COORDINATOR, OFFICE OF RESEARCH SERVICES
Street1*: 433 BOLIVAR ST
Street2: 2ND FLOOR
City*: NEW ORLEANS
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 70112-7021
Phone Number*: 504-599-1533 Fax Number: 504-568-8808 Email: aclesi@lsuhsc.edu

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

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

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Alcohol Use Disorder and Associated Neurological Symptoms of Cognitive Dysfunction and Pain

12. PROPOSED PROJECT

Start Date*	Ending Date*
12/01/2019	11/30/2024

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:	LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO
Duns Number:	7826278140000
Street1*:	
Street2:	
City*:	NEW ORLEANS
County:	Orleans
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	70112-1393
Project/Performance Site Congressional District*:	LA-002

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

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*	ProjectSummary_RC3_CARC_2019_Final.pdf
8. Project Narrative*	
9. Bibliography & References Cited	References_Cited_RC3_CARC_2019.pdf
10.Facilities & Other Resources	
11.Equipment	

Abstract LSUHSC CARC Research Component 3: Alcohol Use Disorder & Associated Neurological Symptoms of Cognitive Dysfunction and Pain

Alcohol use disorder (AUD) is characterized by neurological deficits, negative affective states, and a profound escalation of drinking. The cognitive and behavioral deficits associated with excessive drinking are attributed to functional and persistent changes to neuronal circuitry. Chronic alcohol induced-cognitive impairments are associated with selective central nervous system damage in areas such as the prefrontal cortex (PFC). Excessive alcohol exposure also damages the peripheral nervous system to produce a characteristic neuropathy, and the resulting hyperalgesia (increased pain sensitivity) is hypothesized to potentiate negative reinforcement processes to increase motivation for alcohol. Alcohol use also represents a major exacerbating factor for human immunodeficiency virus (HIV) disease. Even in the post-antiretroviral therapy (ART) era neurocognitive deficits remain prevalent in persons living with HIV (PLWH). HIV-associated neurocognitive disorder (HAND) and co-occurring AUD can exacerbate these deficits. PLWH also suffer from chronic pain, which disrupts physical and emotional function, interferes with ART adherence, and doubles the chance of virologic failure. Pain symptoms in PLWH are associated with specific changes in the brain and correspondingly associates with numerous psychosocial factors in this population, including depression. While cognitive deficits and pain are closely linked in PLWH, few studies have examined the stress-related neurobiological factors that drive these interactions or how alcohol and HIV promote this process. The PFC represents and executes the highest forms of goal-directed behavior, and its function is compromised in motivational disorders such as AUD. As a potential neurobiological correlate of pain and cognitive impairment in PLWH, preclinical studies from our group and others have implicated a functional potentiation of glucocorticoid receptor (GR) signaling in association with excessive alcohol drinking, cognitive dysfunction, and chronic pain. Heightened GR signaling and altered excitability of vulnerable cognition- and pain-related brain areas such as the PFC may thus represent a unifying mechanism contributing to these pathologies. Finally, emerging evidence suggests that Western diets commonly consumed in the United States worsen both neurocognitive and pain symptomatology. Thus, the neurobiological interaction of excessive alcohol drinking, cognition, and pain in the context of Western diet consumption represents a critically underexplored area of HIV research in the public interest. Our overarching hypothesis is that excessive drinking and HIV/ART exposure in individuals consuming a Western diet additively produce cognitive deficits and hyperalgesia in PLWH in association with increased glucocorticoid signaling and hyperexcitability within the PFC. We will examine these factors using a bidirectional translational experimental design incorporating human and nonhuman primate models.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Scott Middle Name Last Name*: Edwards Suffix: Ph.D

Position/Title*:	Assistant Professor
Organization Name*:	LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER - N.O.
Department:	Physiology
Division:	School of Medicine
Street1*:	[redacted]
Street2:	
City*:	New Orleans
County:	
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701120000

Phone Number*: 619-241-3380 Fax Number:

E-Mail*: sedwa5@lsuhsc.edu

Credential, e.g., agency login: [redacted]

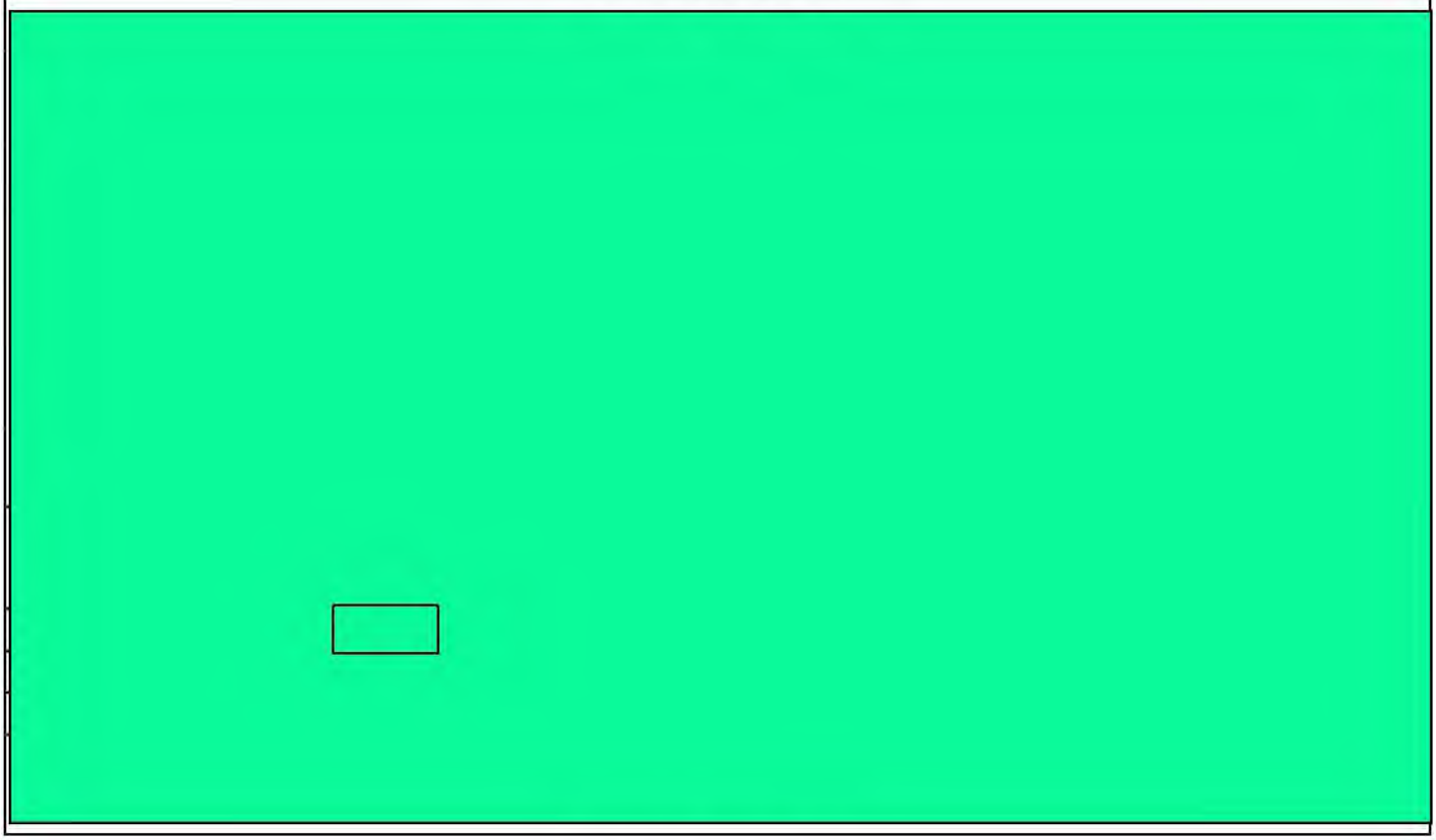
Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

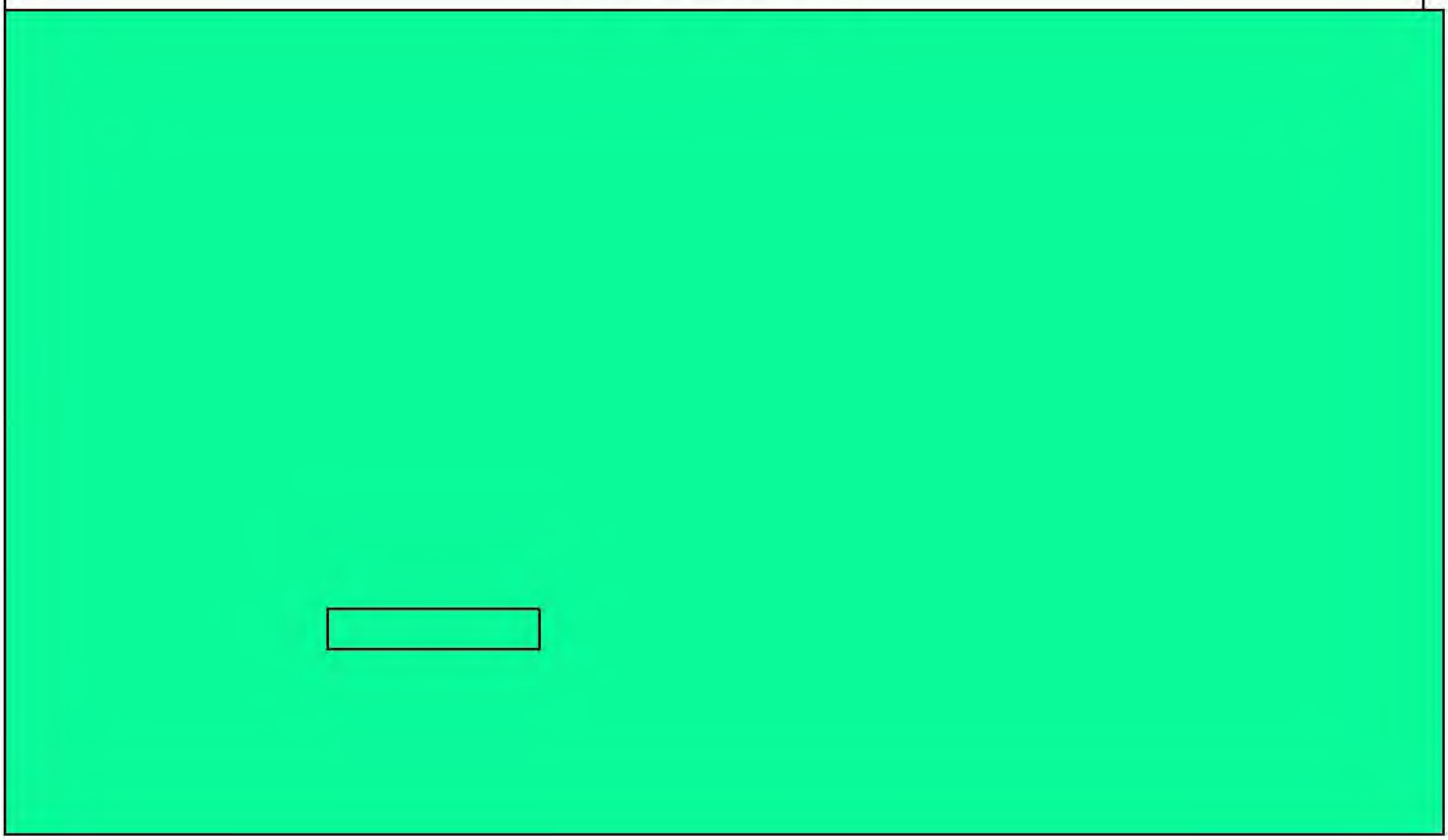
Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person



PROFILE - Senior/Key Person



OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 7826278140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **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.	Dr.	Scott		Edwards	Ph.D	Project Lead					10,769.00	4,631.00	15,400.00
2.					Ph.D	Project Lead					6,209.00	2,670.00	8,879.00
3.					Ph.D	Co-I					10,000.00	4,300.00	14,300.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **38,579.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,579.00**

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

Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **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)

Tracking Number: GRANT12841747
Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	8,666.00
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	
Total Other Direct Costs	**8,666.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**47,245.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	47,245.00	22,205.00
		Total Indirect Costs	**22,205.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (Region 6) Uyen Tran 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**69,450.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	69,450.00

L. Budget Justification*	File Name: P60_RC3_BudgetJustification.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 7826278140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **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.	Dr.	Scott		Edwards	Ph.D	Project Lead					11,092.00	4,769.00	15,861.00
2.					Ph.D	Project Lead					6,395.00	2,750.00	9,145.00
3.					Ph.D	Co-I					10,300.00	4,430.00	14,730.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **39,736.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**39,736.00**

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

Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **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)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,300.00
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	
Total Other Direct Costs	**10,300.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,036.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	50,036.00	23,517.00
		Total Indirect Costs	**23,517.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (Region 6) Uyen Tran 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**73,553.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	73,553.00

L. Budget Justification*	File Name: P60_RC3_BudgetJustification.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 7826278140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **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.	Dr.	Scott		Edwards	Ph.D	Project Lead					11,425.00	4,913.00	16,338.00
2.					Ph.D	Project Lead					6,587.00	2,832.00	9,419.00
3.					Ph.D	Co-I					10,609.00	4,562.00	15,171.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **40,928.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**40,928.00**

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

Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **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*: 7826278140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,609.00
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	
Total Other Direct Costs	**10,609.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**51,537.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	51,537.00	24,222.00
		Total Indirect Costs	**24,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (Region 6) Uyen Tran 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**75,759.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	75,759.00

L. Budget Justification*	File Name: P60_RC3_BudgetJustification.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 7826278140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **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.	Dr.	Scott		Edwards	Ph.D	Project Lead					11,768.00	5,060.00	16,828.00
2.	[redacted]				Ph.D	Project Lead					6,785.00	2,918.00	9,703.00
3.					Ph.D	Co-I					10,927.00	4,698.00	15,625.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **42,156.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**42,156.00**

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

Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **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*: 7826278140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,927.00
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	
Total Other Direct Costs	**10,927.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**53,083.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	53,083.00	24,949.00
		Total Indirect Costs	**24,949.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (Region 6) Uyen Tran 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**78,032.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	78,032.00

L. Budget Justification*	File Name: P60_RC3_BudgetJustification.pdf (Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 7826278140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **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.	Dr.	Scott		Edwards	Ph.D	Project Lead					12,121.00	5,212.00	17,333.00
2.					Ph.D	Project Lead					6,988.00	3,005.00	9,993.00
3.					Ph.D	Co-I					11,255.00	4,840.00	16,095.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **43,421.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,421.00**

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

Funding Opportunity Number: RFA-AA-19-003. Received Date: 2019-04-16T15:14:37.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **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)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 7826278140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER- NO

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	11,255.00
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	
Total Other Direct Costs	**11,255.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**54,676.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	54,676.00	25,698.00
		Total Indirect Costs	**25,698.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (Region 6) Uyen Tran 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**80,374.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	80,374.00

L. Budget Justification*	File Name: P60_RC3_BudgetJustification.pdf (Only attach one file.)

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

Budget Justification: RC3

Personnel:

Scott Edwards, Ph.D., Principal Investigator (effort; calendar months) Dr. Edwards is Assistant Professor (pending promotion to Associate with Tenure, July 2019) of Physiology with dual appointment in the LSUHSC Neuroscience Center of Excellence. He is an expert in the neurobiology of alcohol dependence and associated co-morbidities, with a particular focus on stress-related molecular adaptations. He will devote effort to provide scientific direction and managerial oversight for technical staff and any trainees involved in RC3 aims. Dr. Edwards will work closely with the listed personnel below, and will be responsible for preparation of progress reports, dissemination of data, and all associated regulatory protocols.

, Co-Investigator (effort; calendar months). is the Tom Benson Professor of Neurology with dual appointment in the LSUHSC Neuroscience Center of Excellence and Director of the LSU Pain Mastery Program. is an expert in neurological disorders and pain management, including the direct measurement of pain sensitivity in humans. Drs. Edwards and will work closely together to lead and direct data analysis and dissemination of behavioral findings across humans and non-human primates for RC3, including training of CARC personnel and research publications.

Ph.D., Co-Investigator (effort; calendar months) is a recent faculty hire from Columbia University and will transfer his NIAAA-sponsored R00 to join the Department of Cell Biology & Anatomy at LSUHSC as Assistant Professor in June 2019. is an expert in neurophysiological mechanisms underlying alcohol drinking with a particular focus on prefrontal cortex (PFC) excitability and plasticity, and will lead the PFC electrophysiological and spine density analyses proposed in Aim 3. and Edwards will also work closely together to analyze molecular and neurophysiological data in relation to the behavioral outcomes in NHPs.

Supplementary staff will be composed of existing CARC personnel.

Supplies, travel and other expenses:

Materials and Supplies: Funds for electrophysiological recording and cytology supplies are requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		204,820.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		204,820.00
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		51,757.00
1. Materials and Supplies	51,757.00	
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	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		256,577.00
Section H, Indirect Costs		120,591.00
Section I, Total Direct and Indirect Costs (G + H)		377,168.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		377,168.00

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)

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:

PHS 398 Research Plan

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

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_RC3_CARC_2019_Final.pdf
3. Research Strategy*	Research_Strategy_RC3_CARC_2019_Final.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_animals.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	Letter_of_Support_Scott_CARC_Middleton.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_Plans_CARC_2019_Final.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_Key_Biological_CARC_2019.pdf
Appendix	
12. Appendix	

Specific Aims LSUHSC CARC Research Component 3 (RC3): Neurological Consequences of Alcohol and HIV in the Context of a Western Diet

Alcohol use disorder (AUD) is a chronic, relapsing disease characterized by the gradual emergence of neurological deficits and negative affective states (e.g., cognitive dysfunction, depressive symptoms, pain) and a profound escalation of drinking.[1] The cognitive and behavioral deficits associated with excessive drinking are commonly attributed to persistent neuroadaptations and functional changes to neuronal circuitry, as both former and current AUD patients demonstrate cognitive impairments including deficits in working memory,[2] executive functioning,[3] and impulsivity,[4] all contributing to maladaptive decision-making. These chronic alcohol induced-cognitive impairments are associated with selective central nervous system damage in vulnerable areas such as the prefrontal cortex.[5,6] Excessive alcohol exposure also damages the peripheral nervous system to produce a characteristic neuropathy, and the resulting hyperalgesia (increased pain sensitivity) is hypothesized to potentiate ascending nociceptive circuitry and associated negative reinforcement processes to increase motivation for alcohol.[7]

Alcohol use also represents a major exacerbating factor for human immunodeficiency virus (HIV) disease.[8] Even in the post-antiretroviral therapy (ART) era, HIV infection continues to promote neurological and psychiatric comorbidities that worsen disease outcomes in persons living with HIV (PLWH). Neurocognitive deficits remain prevalent in PLWH, while HIV-associated neurocognitive disorder and co-occurring AUD can exacerbate these deficits.[9] Up to 85% of PLWH also suffer from chronic pain.[10] Pain disrupts physical and emotional function in PLWH, interferes with ART adherence, and doubles the chance of virologic failure.[11] Pain symptoms in PLWH are associated with specific changes in the brain,[12] and pain correspondingly associates with numerous psychosocial factors in this population, including depression and psychological distress.[13] While cognitive deficits and pain are closely intertwined in PLWH,[14] few studies have examined the stress-related neurobiological factors that drive these interactions or how alcohol and HIV promote this process.

The prefrontal cortex (PFC) represents and executes the highest forms of goal-directed behavior, and has thereby attained a central neuroanatomical position in most pathophysiological conceptualizations of motivational disorders, including AUD. As a potential neurobiological correlate of pain and cognitive impairment in PLWH, preclinical studies from our group and others have implicated a functional potentiation of glucocorticoid receptor (GR) signaling in association with excessive alcohol drinking,[15] cognitive dysfunction,[16] and chronic pain.[17] Heightened GR signaling and altered excitability of vulnerable cognition- and pain-related brain areas such as the PFC may thus be a unifying mechanism contributing to these pathologies.[15] Finally, emerging evidence suggests that Western diets (WD) commonly consumed in the United States worsen both neurocognitive[18] and pain[19] symptomatology and disease progression. Thus, the **neurobiological interaction of excessive alcohol drinking, cognition, and pain in the context of WD consumption** represents a critically underdeveloped area of HIV research in the public interest.

Our overarching hypothesis is that excessive drinking and HIV/ART exposure in individuals consuming a WD additively produce cognitive deficits and hyperalgesia in PLWH in association with increased glucocorticoid signaling and hyperexcitability within the PFC. We will examine these factors using a **bidirectionally translational experimental design** incorporating human and nonhuman primate (NHP) models.

Specific Aim 1 will test the hypothesis that **at-risk alcohol use** produces cognitive deficits and hyperalgesia in HIV- (seronegative) humans and NHPs. Aim 1 will employ measures of cognition and nociception in subjects (humans and NHPs) that consume a WD. These behavioral measures will also be compared to peripheral and neurobiological markers of GR signaling examined in Aim 3.

Specific Aim 2 will test the hypothesis that **HIV, simian immunodeficiency virus (SIV), and ART** produce cognitive deficits and hyperalgesia in humans and NHPs. Combinations of data from Aims 1 and 2 will explore interactions between alcohol and HIV/SIV factors. These behavioral measures will also be directly compared to peripheral and neurobiological markers of GR signaling under investigation in Aim 3.

Specific Aim 3 will test the hypothesis that chronic binge alcohol administration to SIV/ART NHPs increases **PFC GR signaling and neuronal hyperexcitability**. Aim 3 will employ *ex vivo* slice electrophysiology techniques to measure intrinsic, active, and synaptic properties of PFC neurons along with measures of dendritic spine morphology and GR system signaling.

Research Strategy LSUHSC CARC Research Component 3 (RC3): Neurological Consequences of Alcohol and HIV in the Context of a Western Diet

SIGNIFICANCE

Alcohol Use Disorder (AUD) & Associated Neurological Symptoms of Cognitive Dysfunction and Pain
Recent epidemiological data using DSM-V criteria indicated that 13.9% of the United States (US) population met the criteria for AUD over the past year.[1] AUD can be conceptualized as a chronic, relapsing disease characterized by the emergence of a constellation of neurological deficits and negative affective states (e.g., cognitive dysfunction, depression, pain) and a profound escalation of drinking.[2] Consequently, the progression from recreational, limited consumption to escalated, at-risk drinking is proposed to involve a motivational transition to negative reinforcement mechanisms. Accordingly, excessive drinking may be driven by attempts to alleviate or mask the negative symptoms of withdrawal. The cognitive and behavioral deficits associated with excessive drinking are commonly attributed to long-lasting neuroadaptations and functional changes to neuronal circuitry, as both former and current AUD patients demonstrate cognitive impairments including deficits in working memory[3], executive functioning,[4] and impulsivity,[5] which all contribute to maladaptive decision-making. These chronic alcohol induced-cognitive impairments are associated with selective central nervous system (CNS) damage in vulnerable areas such as the prefrontal cortex (PFC).[6,7] Excessive alcohol exposure also damages elements of the peripheral nervous system (PNS) to produce a characteristic small fiber neuropathy, and the resulting hyperalgesia (or increased pain sensitivity) is hypothesized to contribute to motivational factors to drink.[8] Indeed, *pain represents a powerful negative subjective experience that can influence reward and reinforcement mechanisms, possibly facilitating the transition to AUD in vulnerable individuals*.[9] Indeed, self-reports of alcohol use specifically for pain management are common.[10] Problem drinkers of both sexes report more severe pain symptoms compared to non-drinkers, and also a higher incidence of using alcohol to manage their pain.[11] Interestingly, use of alcohol to manage pain symptoms predicted worsening alcohol drinking-related health problems (including diabetes) over time in females.[11] Importantly, chronic pain affects approximately 100 million Americans,[12] a number that will likely increase over the next few decades given an aging US population. Moreover, emerging evidence suggests that ***Western diets (WD)*** commonly consumed in the US worsen both neurocognitive[13,14] and pain[15-17] symptomatology and disease progression. Thus, the neurobiological interaction of excessive alcohol drinking, cognition, and pain in the context of WD consumption represents a critical area of research and public health interest.

Interaction of Alcohol Drinking and HIV Infection in the Manifestation of Cognitive Impairment and Pain
Heavy or at-risk alcohol use also represents a major exacerbating factor for human immunodeficiency virus (HIV) disease. As many as 26.9% of adults report regular binge drinking in the US, a behavior that demonstrably worsens HIV-related psychosocial and biomedical outcomes.[18] The neurological consequences of chronic HIV infection span both the peripheral and central nervous system, and despite the success of antiretroviral therapy (ART) to control viral loads, HIV infection and treatment continue to promote neurological and psychiatric comorbidities that worsen disease outcomes and overall quality of life. Neurocognitive disorders are highly prevalent in persons living with HIV (PLWH), while HIV-associated neurocognitive disorder (HAND) and co-occurring AUD can exacerbate these deficits.[19] HAND can include deficits in attention, memory, and executive function that can negatively affect the treatment compliance and disease progression of HIV and AUD. While ARTs have been effective in treating HIV progression and prolonging life, they are not fully protective against the neurocognitive symptoms of HAND and therefore longevity can worsen cognitive impairment.[20] Comorbid heavy alcohol use contributes to neuropsychological symptoms and reduced PFC brain volume.[21] A better understanding of the underlying physiology and neurobiology may reveal additional therapeutic interventions for alcohol and HIV associated cognitive dysfunction. Further progress in AUD and HIV treatment depends on the validity of research models. A bidirectional translational and clinically relevant model for studying AUD and HIV interactions is employed by our Comprehensive Alcohol-HIV/AIDS Research Center (CARC) where nonhuman primates (NHPs) are infected with simian immunodeficiency virus (SIV) and exposed to chronic binge alcohol (CBA) administration. Our previous behavioral experiments in CBA/SIV NHPs demonstrated that these individuals display greater number of errors during a repeated acquisition and performance task following CBA administration compared to SIV-infected non-CBA-treated controls.[22] Our follow-up work has described neuroadaptations in growth factor and neuroinflammatory gene expression in the hippocampus.[23] However, PFC-related neurobiological mechanisms and relative contributions of CBA vs. SIV underlying this neurological deficit remain unknown. In addition, while the distribution of symptoms associated with HAND has shifted (but not been eliminated) in the ART era,[24] HIV-associated neuropathies (HIV-N) have now become the most common neurological disorders associated with HIV infection.[25] HIV-N includes distal sensory polyneuropathy

and antiretroviral toxic neuropathy, with the latter reflecting the neurotoxicity of ART medications themselves. An analysis of patients enrolled in the Central Nervous System HIV Anti-Retroviral Therapy Effects Research (CHARTER) study found that 57% of PLWH suffered from HIV-N.[26] PLWH with HIV-N also have higher rates of depression, anxiety, and insomnia, compared to those without neuropathy,[27] *indicating that peripheral nervous system (PNS) neurological pain symptoms may ultimately transform into CNS pathophysiology including cognitive dysfunction and psychiatric illness*. Indeed, chronic pain is well known to disrupt new learning and executive functioning,[28] which represent unique domains of HAND that have been slow to improve in the ART era.[29] The interaction of ascending pain and central cognitive function is mediated via CNS network nodes and connectivity, including specific cortical and subcortical nuclei (**Figure 1**). Finally, consumption of ***WD*** may further impact PLWH, as central obesity and diabetes is associated with worsened neurocognitive impairment in this population.[30] Further interrogation of specific CNS regional plasticity in relevant preclinical models of HIV infection and ART treatment is necessary to develop better therapeutic strategies for emerging neurological and psychiatric disorders in PLWH consuming WD.

Neurobiological Mechanisms Underlying Alcohol- and HIV-Associated Neurological Disease Risks

The PFC represents and executes the highest forms of goal-directed behavior, and has thereby attained a central neuroanatomical position in most pathophysiological conceptualizations of motivational disorders, including AUD. As one key motivational consequence of cognitive deficits, compromised PFC function leads to a failure of self-regulation processes that can facilitate relapse in individuals suffering from AUD.[31] Evidence suggests a close connection between at-risk alcohol use, dysregulation of physiological stress systems, and compromised PFC function. More specifically, exposure to alcohol produces an allostatic dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis along with heightened forebrain glucocorticoid receptor (GR) signaling that can damage PFC architecture and function.[32] Negative affective states intimately associated with AUD result not only from a dysregulated HPA axis, but also from the inability of a damaged PFC to regulate subcortical stress and reinforcement centers, including the ventral striatum and amygdala. Strong evidence suggests that the neural substrates associated with AUD may also overlap with substrates of emotional aspects of pain processing, where ascending pain pathways connect in frontocortical areas.[33] Specifically, the negative affective and cognitive-disrupting components of pain are regulated by a pathway that includes the PFC, and specifically the anterior cingulate cortex (ACC).[34,35] At the molecular level, neuroendocrine abnormalities appear to closely associate with both executive dysfunction and pain symptomatology, with circulating stress hormones acting within specific brain centers. For example, excessive brain *GR signaling is thought to be a key driver of cognitive deficits and escalation of drinking*, while therapeutic approaches that block excessive GR activity have demonstrated efficacy in treating these two domains at both preclinical and clinical levels.[36,37] Our research group has also demonstrated increased pain sensitivity in alcohol-dependent rats[38] (**Figure 2**), and our ongoing R01-funded studies test contributions of GR signaling to this behavior as systemic administration of GR antagonists reduces alcohol-induced neuropathy.[39] Dysregulation of stress-related GR signaling also likely plays a crucial role in HIV pathology and worsening of cognition-related disease outcomes in PLWH, either as a result of direct damage to the PFC or in close coordination with inflammatory processes.[40] In summary, these findings indicate that PFC dysregulation and potentiation of GR-mediated stress signaling from both excessive alcohol exposure and HIV infection contributes to the emergence of cognitive deficits and pain symptoms. These sequelae may in turn further drive the escalation of alcohol drinking associated with severe AUD, further worsening both alcohol- and HIV-related disease outcomes. Finally, consumption of ***WD*** negative impacts PFC plasticity and PFC-related behaviors.[41]

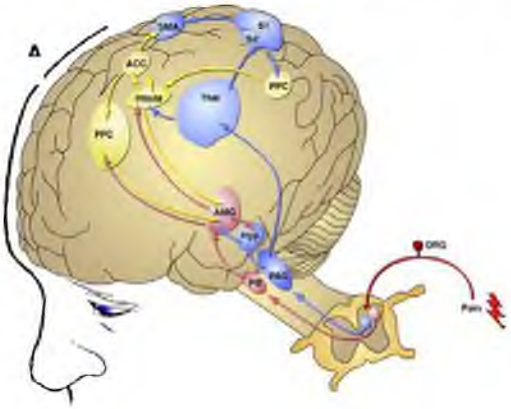


Figure 1 – Excessive alcohol use damages highly specialized CNS cognition- and pain-related circuitry and potentiate ascending nociceptive circuitry to produce enduring cognitive deficits & chronic pain symptoms that are hypothesized to be exacerbated by HIV infection. This proposal seeks to investigate specific neuroadaptations in frontocortical glucocorticoid receptor (GR) signaling and hyperexcitability in relation to cognitive and nociceptive behaviors in a rhesus macaque model of alcohol/HIV comorbidity. Our primary hypothesis focuses on subregions of the prefrontal cortex (lateral PFC & anterior cingulate cortex, ACC). From Egli, Koob, & Edwards (2012).

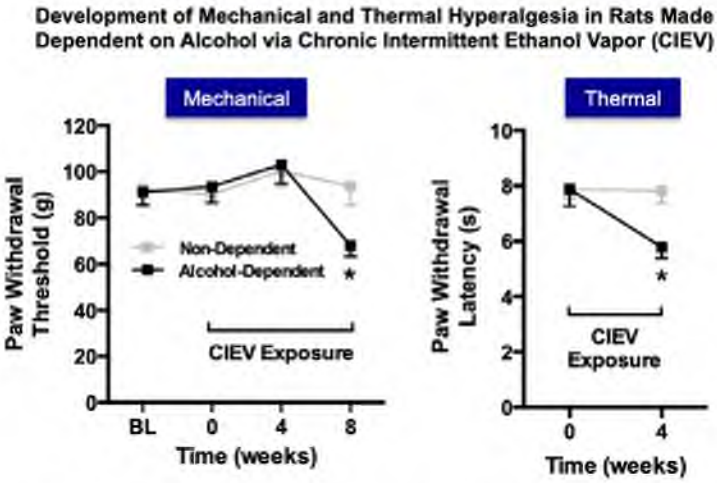


Figure 2 – Hyperalgesia symptoms are manifest in rats made dependent on alcohol via chronic, intermittent ethanol vapor (CEIV) exposure. CIEV exposure in rats and CBA administration in NHPs produce similar peak blood alcohol levels with intermittent periods of withdrawal when hyperalgesia may be tested. Hyperalgesia is detected via Hargreaves thermal tests (Roltsch Hellard et al., 2016) and von Frey mechanical tests (Edwards et al., 2012). *p<0.05 significantly lower paw withdrawal thresholds (mechanical) or latencies (thermal) via *post hoc* tests following significant group x time interaction by ANOVA, n=8-12/group.

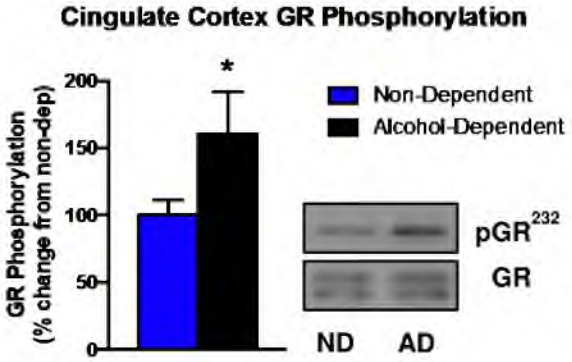
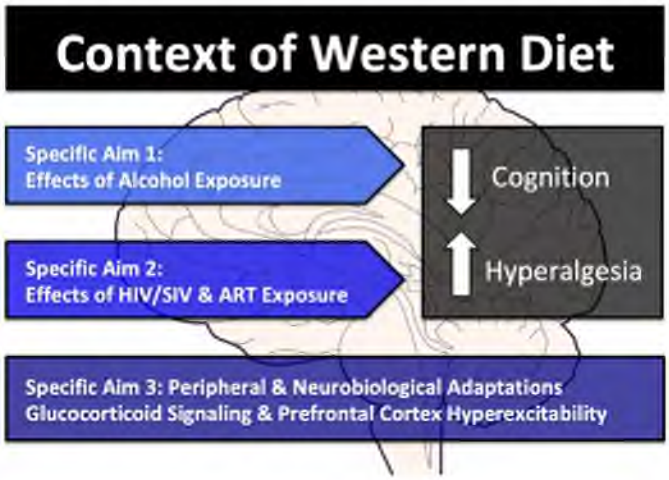


Figure 3 - Alcohol-dependent male rats exhibit increased GR phosphorylation at serine 232 in the cingulate cortex relative to non-dependent controls (*p<0.05, n=9-14/group) At the molecular level, the transcriptional activity of GR is directly controlled via phosphorylation at this specific residue (Adzic et al., 2009). This proposal will examine whether this change translates into an NHP model.

Summary, Hypotheses, and Scientific Premise

There is an urgent programmatic need to understand the neurobiological interaction of at-risk alcohol drinking, cognition, and pain in PLWH. Based on the multifaceted deleterious effects of WD consumption and the relevance of this factor on our New Orleans Alcohol Use in HIV (NOAH) longitudinal patient population and their specific comorbidities, we propose to examine the interaction of alcohol and HIV disease within the context and under the influence of a WD and WD-related variables. Our overarching hypothesis is that excessive drinking and SIV/ART exposure in individuals consuming a WD additively produce cognitive deficits and hyperalgesia in PLWH in association with increased GR signaling and hyperexcitability within the PFC. We will examine these factors using a **bidirectionally translational experimental design** incorporating human and nonhuman primate (NHP) models. Indeed, our published and preliminary data across rodent and NHP models and early data from the NOAH cohort in association with our CARC pilot project support further exploration of these aims. The proposed approach uses advanced techniques, and leverages our established NHP model of HIV infection and the parallel NOAH clinical longitudinal study. The rich scientific environment and outstanding infrastructure of the LSUHSC Comprehensive Alcohol-HIV/AIDS Research Center (CARC) confers exceptional synergy to conduct bidirectional translational studies of alcohol's impact on vital health outcomes in PLWH. The expected results will have a profound impact on identification of the underlying mechanisms of alcohol-associated neurological comorbidities and will identify relevant targets for future therapeutic interventions.



Innovation

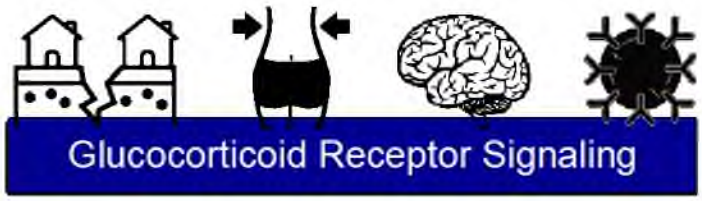
1. This RC employs an innovative bidirectional translational design (see **Experimental Group Timelines** at the end of the Research Strategy) that takes advantage of the respective strengths of longitudinal human patient cohorts and NHP animal models across all three aims. Here, we will implement this strategy to investigate two key neurological behaviors that manifest in both individuals suffering from AUD and PLWH: cognitive dysfunction and enhanced pain sensitivity (measured via surveys in PLWH as well as direct tests in both populations of two distinct nociceptive modalities: mechanical and thermal hypersensitivity). Tracking of cognitive and nociceptive data over time may reveal important associations between how these symptoms progress in accordance with at-risk drinking behaviors in PLWH. Importantly, we will measure alcohol drinking by a variety of both interview (e.g., Alcohol Use Disorder Identification Test (AUDIT), Timeline Followback (TLFB)) and physiological (e.g., PEth) measures.

2. Pain is most commonly conceptualized as a peripheral or spinal pathology, although studies from our lab and others have demonstrated a functional "centralization" of ascending nociceptive signaling within what are traditionally known as brain stress and cognition centers. This proposal seeks to continue the recent momentum toward understanding **central brain** mechanisms of pain and associations with other neurological (cognitive dysfunction) and psychiatric (AUD) disorders. Findings from this work are expected to promote the development of new treatments that target the affective/motivational components of pain (and pain relief). Moreover, while the popular media has primarily focused on the prescription opioid abuse epidemic, we believe that too little attention has been paid to individuals who may abuse alcohol to manage their pain. Ultimately, we predict that this work could have broad implications for our understanding of the negative reinforcing properties of persistent pain and parallel abuse of multiple substances in PLWH.

3. Glucocorticoid signaling is physiologically vital, with receptors present in most organ systems. However, the regulation of GR activity is highly tissue-specific, and a better understanding of GR activity in the periphery vs. CNS (including specific brain areas) is warranted and an innovative feature of our proposal. We hypothesize a dysregulation of GR in cognition- and pain-related areas, while changes in peripheral (PBMC) GR phosphorylation may serve as a readily accessible biomarker of these conditions in humans and NHPs. Moreover, GR signaling could represent a **critical nexus linking separate disease mechanisms across research components (Figure 4)**, given the principle element of glucocorticoid function in psychosocial stress (RC1), metabolism (RC2), neurological disorders (RC3), and immune system regulation (RC4).

4. Aim 3 of our proposal seeks to maximize the utility of the NHP brain tissue by proposing mechanistic molecular analyses in parallel to structural and functional investigations of spine density and synaptic hyperexcitability. These cellular and molecular variables will be directly correlated back to clinical behavioral variables under investigation in Aims 1 and 2 of RC3, and are also expected to integrate into our interpretation of disease mechanisms under investigation in other RCs (**Figure 4**).

Figure 4 – Glucocorticoid receptor (GR) signaling integrates and impacts virtually all physiological systems. Alterations in GR activity may represent a critical intersection linking RC1 (psychosocial stress), RC2 (metabolic risk), RC3 (neurological damage), and RC4 (immune system dysregulation).



Experimental Approach

Specific Aim 1: To test the hypothesis that at-risk alcohol use will produce cognitive deficits and hyperalgesia in HIV- (seronegative) humans and nonhuman primates (NHPs).

Aim 1 Rationale: Chronic and excessive drinking associated with AUD can produce cognitive deficits and a characteristic painful small fiber neuropathy in vulnerable individuals, although the physiological and neurological mechanisms underlying these complications of AUD remain largely uncharacterized. The purpose of Aim 1 is to measure these neurological variables in seronegative humans with at-risk alcohol use in a longitudinal design and in seronegative NHPs receiving chronic binge alcohol (CBA) administration and fed a WD (see **Experimental Group Timelines** at the end of the Research Strategy and **Statistical Analysis** section for number of experimental subjects and justification). Our hypothesis is that at-risk drinking (in the NOAH cohort)

and CBA administration (in NHPs) produces significant cognitive deficits and hyperalgesia. We will examine cognition in humans via the Montreal Cognitive Assessment (MoCA, **Figure 5**) and in NHPs via the Novel Object Recognition Task (NOR, **Figure 6**). Pain assessments will be performed in humans via the Brief Peripheral Neuropathy Screening Tool (BPNS), while pain sensitivity will be directly measured in both humans and NHPs via Hargreaves (thermal) and von Frey (mechanical) sensitivity tests (**Figure 2**). Harry Gould, MD (Co-I of RC3) is an experienced neurologist and pain specialist who regularly conducts these same tests in his clinical practice, and will train CARC scientists to reliably conduct these procedures. We will also incorporate measures of spinal nociceptive reflexes in NHPs via tail dip tests in an attempt to better differentiate spinal vs. potentially supra-spinal changes in nociception.

AUDIT Score Category	% with MoCA Score <26
≥ 16 (High Risk)	100.0%
8-15 (Medium Risk)	89.5%
< 8 (Low Risk)	81.5%

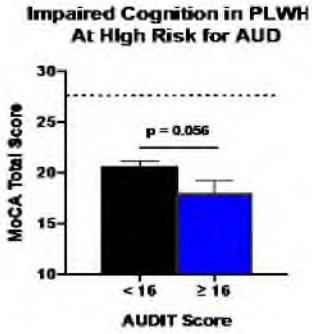


Figure 5 – (Left) Higher Alcohol Use Disorder Identification Test (AUDIT) scores (indicating greater AUD risk) is associated with greater cognitive impairment (MoCA scores < 26) in PLWH. Data are taken from a pilot convenience sample of PLWH enrolled in the ongoing NOAH longitudinal study (n=126, p=0.052). (Right) Individuals at high risk for AUD exhibit lower MoCA scores compared to those at medium or low risk (p=0.056). The dashed line indicates the average MoCA score in healthy individuals (27.5), suggesting that HIV infection may also be acting to lower scores in our current cohort.

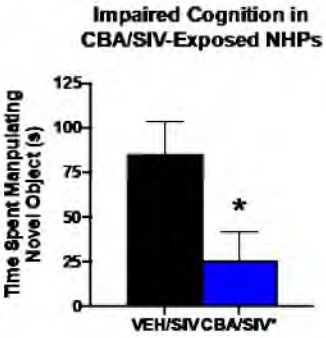


Figure 6 – In close accordance with pilot data from the NOAH cohort, female CBA/SIV-exposed NHPs display cognitive impairment as indexed by reduced time spent with a novel object on day 2 of the NOR task (p=0.043). Data are taken from female NHPs from the previous iteration of this grant and under the auspices of our CARC pilot grant awarded within the last cycle. It remains unclear how SIV/ART exposure alone alters performance in this task, although this will be investigated in Aim 2 of this proposal.

Aim 1 Expected Outcomes & Interpretation of Results: The primary outcome measure under investigation in Aim 1 is cognition. We expect that humans exhibiting high AUDIT scores (AUDIT ≥ 16) will also display lower MoCA scores (indicative of a cognitive deficit, our primary outcome variable) compared to individuals with low AUDIT (<8). Indeed, preliminary data gathered from our pilot study using the current NOAH cohort indicate this relationship exists in PLWH (**Figure 5**), although this relationship may be influenced by HIV infection and/or ART therapy (see Aim 2). In accordance with these findings, previous work from our Center has revealed cognitive deficits in SIV-infected NHPs exposed to CBA.[22] This study incorporated an operant task that revealed deficits only when animals were intoxicated with alcohol. Our recent pilot study adopted a more tractable model of cognitive function that employs the NOR task. Here, we discovered that CBA-treated female NHPs exhibit a decreased time spent with the novel object, indicative of a cognitive deficit (**Figure 6**). Importantly, these findings were observed one day following the last alcohol administration (i.e., not under a state of alcohol intoxication), a condition that is likely more commensurate with investigations of our NOAH patient population, and more likely to reflect ongoing cognitive deficits between alcohol exposures (or drinking bouts in humans). We will also examine latency to approach the novel object as an additional outcome measure of cognitive deficit, with CBA-treated animals expected to display an increased latency. Our previous work has also described the development of enhanced pain sensitivity, or hyperalgesia, in alcohol-dependent rats[38] that models painful neuropathic symptoms observed in individuals with a history of excessive drinking.[42] For the current proposal, we expect that individuals exhibiting high AUDIT scores (AUDIT ≥ 16) will also score higher on the BPNS neuropathy scale compared to individuals with low AUDIT (<8). To complement survey data, we will also conduct more empirical Hargreaves and von Frey assays (measures of thermal and mechanical supra-spinal nociceptive sensitivity, respectively). We expect subjects with higher AUDIT to display reduced mechanical thresholds (measured in grams) and reduced thermal latencies (measured in seconds). This proposal also extends these findings by examining these relationships in an NHP model, where we will conduct tail dip assays (measure of spinal nociceptive reflexes) along with Hargreaves and von Frey assays (measures of spinal and supraspinal

nociceptive processing). In these procedures, we expect CBA-exposed NHPs to display decreased tail withdrawal latencies (tail dip test), decreased paw withdrawal thresholds (von Frey test), and decreased paw withdrawal latencies (Hargreaves test), all indicative of hyperalgesia.

Aim 1 Limitations and Alternative Approaches: In addition to AUDIT, we will collect several other measures of at-risk drinking using both survey (e.g., TLFB) and physiological (e.g., phosphatidylethanol, PEth) measurements. Our preliminary data indicates a high number of NOAH patients with low MoCA scores in general (**Figure 5**). This may reflect that our current NOAH cohort consists of PLWH, and HIV infection may be driving down baseline scores (see Aim 2). Therefore, we plan on retaining the MoCA analysis for this proposal to test this hypothesis. In case we observe floor effects in these analyses, there is a simpler version of the MoCA available to administer[43] (MoCA-Basic). Based on our preliminary NHP data, we do not expect any procedural issues with the NOR task. A limitation of this approach is that we are relying on a single behavioral assay of cognition. To remedy this, we are looking at multiple readouts of cognitive function within the task (i.e., latency to approach novel object & time spent with novel object, along with ratios of novel object vs. familiar object recognition). Our premise for analyzing alcohol-related changes in pain sensitivity in humans and NHPs is largely driven by our work in rodents.[38] We expect this proposal to be a vital and translational extension of our rodent work into humans and NHP models. We have proposed an analysis of both thermal and mechanical nociceptive sensitivity in the case that changes are present in only one sensory modality. Importantly, we are able to use most of the same testing equipment (i.e., von Frey and Hargreaves) across humans and NHPs to measure pain sensitivity, as this equipment is routinely used across species (from rodent to human). We therefore expect few if any problems measuring nociceptive sensitivity in higher species based on the substantial combined experience of Dr. Gould and our CARC NHP behavioral scientists. We will collaborate with Dr. Liz Simon (Co-Director of the Experimental and Analytical Core) to perform tail dip procedures in NHPs. Operant tasks[22] remain a valuable alternative cognitive task for us, and may be utilized in the case that we do not see significant effects using NOR in early experimental cohorts, or as guided by MoCA subscore data in our NOAH cohort.

Specific Aim 2: To test the hypothesis that HIV/SIV and ART will produce cognitive deficits and hyperalgesia in humans and NHPs.

Aim 2 Rationale: Similar to the deleterious effects of alcohol on neurological sequela, HIV infection can also produce profound cognitive deficits and painful neuropathic symptoms. Previous CARC studies focused on examinations of the combined effects of HIV/SIV and alcohol on behavioral and physiological outcomes, making it difficult to tease out the relative contributions of these two factors. In this proposal, Aim 2 is designed to specifically examine the effects of HIV/SIV (in combination with ART medication exposure) on these behavioral outcomes, independent of alcohol history or exposure.

Aim 2 Expected Outcomes & Interpretation of Results: Importantly, our 2x2 experimental design across humans and NHPs will allow for the examination of the main effects of alcohol, the main effects of HIV/SIV/ART, as well as interactions between these factors (expected to be additive) on our primary outcome variable (cognition) and main exploratory variable (pain). We first expect that PLWH will display lower MoCA scores (indicative of a cognitive deficit) compared to seronegative controls. Our preliminary data (**Figure 5**) indicates a greater likelihood of cognitive deficits in PLWH with higher AUDIT scores, although a high percentage of all our subjects scored <26 (with scores ≥26 considered normal[44]). This may indicate (and we hypothesize) that HIV/ART exposure itself acts as an independent factor to produce cognitive deficits. We also expect that PLWH will exhibit neuropathic symptoms (indicated by the BPNS) and reduced thermal and mechanical nociceptive thresholds (indicative of hyperalgesia or increased pain sensitivity). In our NHP model, we expect to recapitulate these behavioral findings, with SIV+ NHPs displaying reduced time spent manipulating a novel object in NOR tests (indicative of a reduced ability to discriminate a novel from a familiar object). We also hypothesize that SIV+ NHPs will display hyperalgesia as indicated by tests of spinal nociception (reduced tail dip latency) and supraspinal nociception (reduced paw withdrawal thresholds in von Frey tests and reduced paw withdrawal latencies in Hargreaves tests).

Aim 2 Limitations and Alternative Approaches: Many pitfalls and alternative approaches are similar to Aim 1 as we are measuring the same primary (cognition) and exploratory (pain) outcome variables but with an additional factor under investigation in Aim 2. However, unique HIV-related aspects of the expected neurological deficits (cognitive deficits and neuropathy) may require a more detailed investigation between these factors. For example, the severity of cognitive impairments associated with HAND has been reduced in the ART era. Indeed, fewer PLWH exhibit HAND, but the number with asymptomatic neurocognitive impairment (ANI) has increased.[45]

Perhaps most importantly, the profile of cognitive impairment has also changed in the ART era. The percentage of PLWH presenting with impairment in verbal domains, speed of information processing, and motor coordination domains have decreased, while the percentage of those with impaired learning and executive function domains have increased.[29] Thus, a more detailed analysis of MoCA subscores specifically relating to these cognitive components (versus the aggregate score) may be warranted. Importantly, these shifts in specific cognitive deficits may also reflect differential vulnerabilities of brain regions to HIV-associated neuropathology, which will inform NHP brain investigations in Aim 3. Somewhat similarly, the NOR task appears able to measure cognitive deficits in NHPs exposed to CBA/SIV/ART, but cognitive deficits produced by SIV/ART vs. CBA may not be identical. To remedy this limitation, we will use any newly discovered MoCA subscore data differences in our NOAH human cohorts to guide the selection of future cognitive tests for our NHPs (i.e., tests that more precisely match on to the specific cognitive deficit categories observed in humans, such as executive function deficits).

Specific Aim 3: To test the hypothesis that at-risk alcohol exposure and SIV/ART exposure increases frontocortical GR signaling and neuronal hyperexcitability.

Aim 3 Rationale: Heightened GR activity in the PFC is hypothesized to drive the manifestation of neurological deficits (cognitive dysfunction and pain hypersensitivity) associated with AUD,[36] typically in association with frontocortical hyperexcitability.[46,47] In addition, HIV infection and associated inflammatory processes may intimately interact with stress-regulated glucocorticoid signaling to promote both systemic and CNS-related pathology.[40] Aim 3 will examine the neurobiological correlates of cognitive dysfunction and pain sensitivity in NHPs with a focus on PFC GR signaling and neuronal excitability (**Table 1**). On the morning of necropsy at the end of all treatments (see **Experimental Timeline** at the end of the Research Strategy), brains will be extracted and divided into two hemispheres for further analysis. The right hemisphere will be snap frozen in isopentane and stored at -80 degrees until later dissection for western analyses of GR-related targets (**Table 2**). The left hemisphere will be prepared by Dr. Mike Salling (RC3 Co-I) for *ex vivo* electrophysiological recordings (conducted on the day of necropsy) and spine density analyses (see Methods). Due to the known role of the primate lateral prefrontal cortex (LPFC) in cognitive function[48] and anterior cingulate cortex (ACC) in both cognition and pain,[35] we hypothesize that CBA/SIV/ART exposure alters structural and electrophysiological properties of neurons within these areas. We further expect to discover an increase in frontocortical GR signaling (**Table 2**) in CBA/SIV/ART-exposed NHPs, and include multiple protein and phosphoprotein measures relating to positive and negative mediators of GR activity. Finally, we will measure longitudinal GR system changes in PBMCs collected from the NOAH cohort and NHPs to better understand the relationship between the evolution of peripheral glucocorticoid signaling changes and neurological disease processes. Analysis of peripheral GR signaling may also further relate to mechanisms under investigation in other aims. Importantly, all molecular and neurophysiological data collected in Aim 3 will be directly compared to behavioral outcomes measured in Aims 1 and 2 above. Indeed, our preliminary findings from our CARC pilot study suggest possible relationships between enhanced GR signaling and cognitive deficits (**Figures 8 & 9**).

Primary Brain Regions Investigated	Outcome Measures
Lateral Prefrontal Cortex (LPFC)	GR signaling, Ex Vivo Electrophysiology, Spine Density Analysis
Anterior Cingulate Cortex (ACC)	GR signaling, Ex Vivo Electrophysiology, Spine Density Analysis

Table 1 - In addition to the initial target regions listed above, we plan to archive additional NHP brain regions that correspond to CARC-related interests (e.g., hypothalamus, hippocampus, central amygdala), with a full list to be approved by the CARC IC before dissections begin. We will analyze PBMC and brain region-specific adaptations in GR signaling components (total GR, pGR^{S232}, pGR^{S246}, SRC-1, NCOR1, HSD11B1) and will have sufficient tissue available to explore alternative systems linked to other RCs, such as metabolic/insulin signaling and neuroinflammatory signaling.

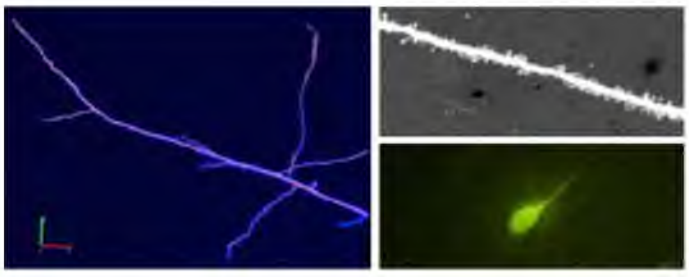


Figure 7 – **(Left & Top)** Whole cell recordings with 0.4% biocytin included in patch pipette allow for neuronal morphology and dendritic spine analysis to be quantified using Neuolucida 360 software (Mouse PFC shown). (**Bottom**) Slices can also be fixed and post processed for neuronal morphology using injections of Lucifer Yellow in additional brain regions (NHP LPFC shown). Images from RC3 Co-I Dr. Mike Salling.

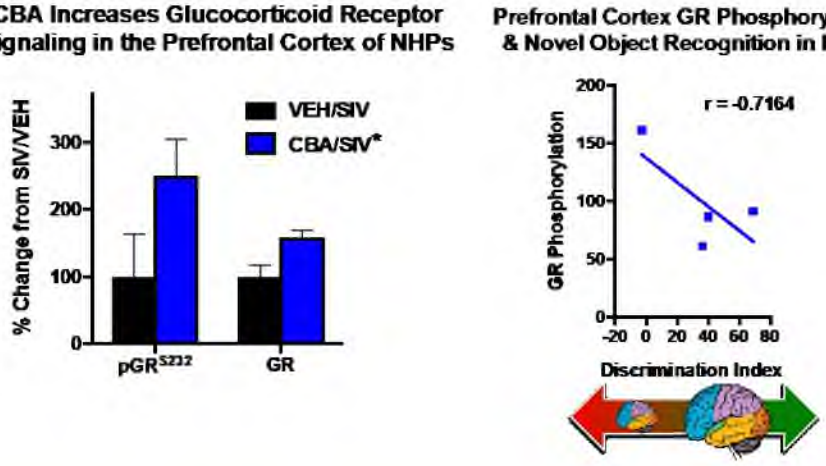


Figure 8 – (**Left**) Female SIV/CBA-exposed NHPs (n=6/group) exhibit significant increases in GR signaling in the lateral PFC compared with SIV/VEH NHPs (n=3/group) ($F_{1,14}$ = 5.283; p =0.0375; main effect of CBA on both total GR and pGR^{S232}). Since increases in both pGR^{S232} and GR were observed, each of these proteins were normalized to tubulin as a loading control. These neuroadaptations may relate in part to observed decreases in cognitive performance indicated in **Figure 6** above. (**Right**) A separate pilot sample of male NHPs displayed a negative correlation between GR^{S232} phosphorylation (pGR/GR ratio) and cognitive performance in the NOR task. Discrimination index refers to the difference score between latencies to interact with a novel vs. familiar object on test day, with a greater score indicating a shorter latency to interact with the novel object and better cognitive performance. These data provide rationale for our hypothesis that enhanced PFC GR signaling promotes cognitive deficits.

Molecular/Physiological Target	CBA Exposure	SIV/ART Exposure	CBA + SIV/ART Exposure
GR	Increase	Increase	Additive Increase
pGR^{S232} (Positive Regulation)	Increase	Increase	Additive Increase
pGR^{S246} (Negative Regulation)	Decrease	Decrease	Additive Decrease
SRC-1 (Positive Regulator)	Increase	Increase	Additive Increase
NCOR1 (Negative Regulator)	Decrease	Decrease	Additive Decrease
HSD11B1 (Local Cort Synthesis)	Increase	Increase	Additive Increase
PFC Excitability	Increase	Increase	Additive Increase
PFC Spine Density	Decrease	Decrease	Additive Decrease

Table 2 – Expected outcomes for molecular and physiological variables in Aim 3 NHP analyses. We hypothesize a general increase in GR signaling in the PFC (both lateral PFC and anterior cingulate cortex) in association with synaptic hyperexcitability and decreased neuronal spine density. Bath application of alcohol is expected to reduce this hyperexcitability, potentially mimicking the use of alcohol as a self-medication strategy in PLWH and/or AUD. We will also conduct analyses of GR signaling in PBMCs in both humans and NHPs to uncover potential peripheral biomarkers of cognitive dysfunction and pain in the groups under investigation.

Aim 3 Expected Outcomes & Interpretation of Results: For Aim 3, our primary outcome measure is protein- and phosphoprotein-level neuroadaptations in GR signaling in cognition- & pain-associated frontocortical areas (LPFC & ACC). We expect to discover net increases in GR-related signaling in both CBA- and SIV/ART-treated animals (**Table 2**), including increases in markers of positive regulators of GR signaling and decreases in markers of negative regulators of GR signaling. We further expect to reveal additive effects of CBA and SIV/ART to potentiate GR-related signaling. For electrophysiological recordings, we will collect LPFC and ACC brain slices taken from the left hemisphere of animals on the morning of necropsy to measure *ex vivo* intrinsic, active, and synaptic properties (inhibitory post-synaptic currents; IPSC and excitatory post-synaptic currents; EPSCs) from neurons both before and after acute bath application of alcohol (20 and 50 mM). Based on the known relationship between GR signaling and PFC excitability,[47] we hypothesize that CBA/SIV/ART animals will exhibit increases in neuronal excitability (input resistance and EPSCs) that will be reversed following the bath application of acute alcohol (see **Figure 9**). Reduction of PFC hyperexcitability by alcohol may underlie the use of alcohol self-medication strategies in AUD. In addition to neuronal recordings, neuronal morphology will be examined from the LPFC and ACC of CBA/SIV/ART animals (see Methods). These data would complement electrophysiological data collected by revealing changes in dendritic spine density that may regulate intrinsic and synaptic activity, or correlate with observed cognition and pain symptoms in NHPs. Importantly, SIV-infected animals recapitulate many aspects of the organ pathology associated with HIV including neuropathologies like reductions in cortical dendritic spine density[49] seen in PLWH.[50] We hypothesize that CBA/SIV/ART animals will display the greatest spine density loss among groups (see **Table 2**). We will also collect additional tissue for spine density measurement in larger neuronal populations across layers.

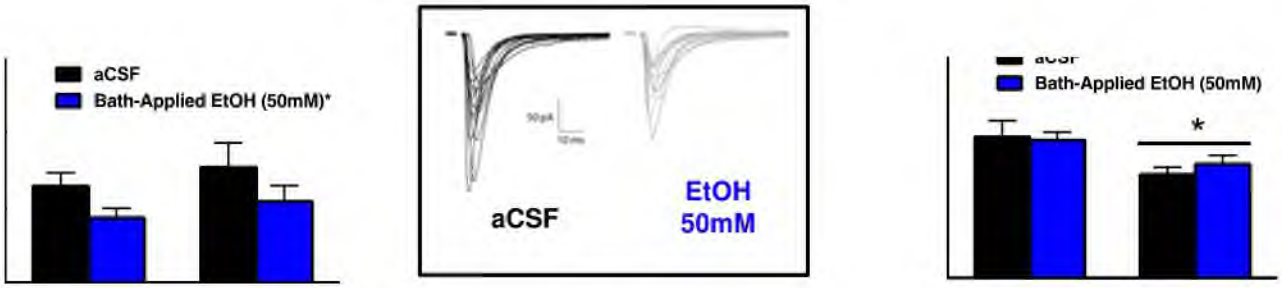
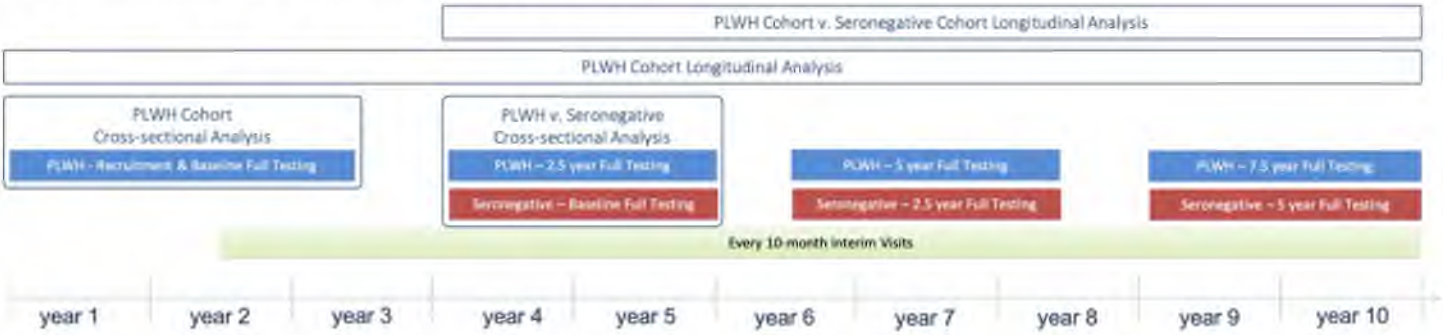


Figure 9 – Electrophysiological recordings from our CARC pilot study in female NHP. (**Left**) Bath-applied alcohol (50mM ethanol, EtOH) reduces EPSC amplitudes measured in *ex vivo* recordings from female NHP caudate taken on the morning of necropsy (n=10-19 recordings/group; $F_{1,56} = 5.349$; p=0.0244; main effect of bath-applied alcohol to decrease excitability). These results align with our published findings of NHP cognitive deficits in an operant task when animals are in a state of alcohol intoxication (Winsauer et al., 2002). (**Center**) *Ex vivo* EPSC amplitude recordings following bath application of aCSF (control) or 50mM ethanol (modeling alcohol intoxication). (**Right**) CBA/SIV exposure decreases paired-pulse ratio in female NHPs, further indicating striatal deficits (n=5-9 recordings/group; $F_{1,23} = 7.094$; p=0.0139; main effect of CBA/SIV to decrease PPR). The current proposal will examine the regulation of NHP prefrontal cortex (PFC) excitability by CBA and SIV in the context of a WD, and compare to changes in cognitive and pain behaviors and glucocorticoid signaling in the PFC. Here, we hypothesize that increased PFC GR phosphorylation/activity will associate with PFC hyperexcitability (see **Table 2**) that will be reduced via bath EtOH, modeling drinking as a self-medication strategy in PLWH and/or AUD.

As a clinical correlate, we will examine GR signaling in PBMCs collected longitudinally from NOAH subjects. We expect that enhanced GR signaling in the periphery will act as a readily accessible biomarker of pain and cognitive dysfunction across individuals. We will perform cross-sectional comparisons as well as longitudinal analyses that will allow us to describe related changes over time between important variables (e.g., cognition, pain, AUDIT & other measures of at-risk drinking). Importantly, this analysis will also allow for important mechanistic bridges to other RCs, which may provide alternative interpretations of collected data. For example, evidence suggests that PLWH exhibit elevated levels of stress (RC1), yet also display glucocorticoid resistance at the level of mononuclear cells,[51,52] leading to enhanced inflammation that may tie into RC4 of this proposal. Enhanced inflammation (in the form of elevated IL-2 and IL-4 levels) has also been demonstrated to alter GR binding affinity.[53] Other studies have discovered that the HIV structural protein, Vpr, is a co-activator of GR and may promote glucocorticoid hypersensitivity associated with insulin resistance (RC2).[54] We believe that our focus on GR phosphorylation and GR co-regulatory proteins will shed additional light on these mechanisms, particularly in association with discoveries made by other RCs within this renewal proposal.

Aim 3 Limitations and Alternative Approaches: Based on the generation of a NHP brain tissue bank, we will have the opportunity to explore alternative mechanisms related to pain and cognition, including endogenous opioid signaling. In that specific case, we would switch to qPCR analyses in separate sample dissection sets, as no reliable antibodies exist for any of the opioid receptors. Our group is also well experienced in measuring glutamate channel subunit phosphorylation (e.g., $GluR1^{S845}$, $NR1^{S897}$, which may closely relate to alterations in neuronal excitability and/or plasticity). There is evidence for both genomic and non-genomic GR regulation of PFC glutamate neurotransmission; as such, we may investigate changes in membrane-associated vs. nuclear-localized GR in separate sample dissection sets via subcellular fractionation methods. At a minimum, we anticipate dissecting serial thick sections for Western and qPCR analyses, in addition to other future analyses as desired (e.g., enzyme activity assays). Residual brain slices (minus the dissected material) will remain catalogued and archived at -80 degrees and available for future dissection and dissemination to other RCs and/or outside collaborators. As mentioned above, in the future, we feel that additional cognitive tasks could be administered to macaques via touchscreen interfaces, including delayed response (DR) and delayed match to sample (DMS) tests. A recent study found that DMS performance in female macaques was inversely correlated to *Hsd11b1* and *Nr3c1* (GR) hippocampal gene expression,[55] indicative of the deleterious role of cortisol/GR signaling on cognitive behaviors. Importantly, these same tasks can readily be administered to human subjects for cross-species comparison. A significant strength of the current proposal is the archiving of macaque brain tissue for future alternative cellular and molecular analyses. Future directions may also incorporate *ex vivo* electrophysiological testing of therapeutic strategies targeting GR signaling and hyperexcitability. For example, object recognition memory impairments in alcohol-dependent mice are attenuated following systemic administration of the GR antagonist mifepristone,[56] and we could apply mifepristone to slices to examine contributions of (likely non-genomic) GR activity to electrophysiological features. In addition to [redacted] several CARC collaborators at our institution have experience in recording from NHP tissue, including [redacted] (see Letter of support); this will allow for recording from multiple regions on the same day.

Experimental Group Timelines & RC3 Methods



Montreal Cognitive Assessment (MoCA): The MoCA is a widely utilized screening assessment for detecting cognitive impairment. It is a single-page, 30-point test administered in approximately 10 minutes. MoCA assesses the following cognitive domains: memory recall, visuospatial ability, executive function, attention, concentration, working memory, language, and orientation to time and space. An aggregate score of 26 or over is considered to be normal. Analysis of subscores may provide addition insight into specific deficits. **Novel Object Recognition (NOR) Task:** NOR is a highly validated test for recognition memory that can be used to test the negative effects of certain other factors on memory performance. Each NHP is presented with two similar objects during the first session, and then one of the two objects is replaced by a new (novel) object during a second test session. The amount of time taken to explore and/or manipulate the new object provides an index of recognition memory. Latency to manipulate the novel vs. familiar object can also be expressed as a discrimination index related to recognition memory. **Brief Peripheral Neuropathy Scale (BPNS):** The BPNS tool assesses subjective and objective findings consistent with PN and was developed and validated by the National Institutes of Health–funded AIDS Clinical Trials Group. Patients are asked to rate presence and severity of symptoms, using a scale of 1 (mild) to 10 (severe) for each leg separately. Symptoms include pain, aching, or burning in feet and/or legs; "pins and needles" in feet and/or legs; and numbness in feet and/or legs. The single highest of the six scores (three for each leg) is then converted to a subjective peripheral neuropathy grade as follows: symptoms absent = grade 0, score of 1–3 = grade 1, score of 4–6 = grade 2, and score of 7–10 = grade 3. Symptoms do not have to be bilateral to be graded as ≥ 1. Objective findings included in the BPNS are loss of vibration perception and abnormal ankle deep tendon reflexes. Vibration perception is evaluated using a 128-Hz tuning fork, maximally struck and applied at the great toe distal interphalangeal joint of each foot. Vibration sense is defined as normal for a vibration felt for > 10 seconds, mild loss for a vibration felt for 6–10 seconds, moderate loss for a vibration felt for ≥ 5 seconds, and severe loss for no feeling of vibration. Ankle reflexes are defined as absent, hypoactive, normal, hyperactive, or clonus. **Hargreaves Method**: To assess thermal sensitivity, each hand (human) or hindpaw (NHP) is stimulated using a halogen heat source from a Hargreaves apparatus (Stoelting, Wood Dale, IL). A 20-second cut-off is employed to prevent tissue damage in non-responsive subjects. On test days, subjects are allowed 10 min to acclimate to the testing room/apparatus, which for NHPs consists of a chair and a glass floor suspended 30 cm from the ground. For humans, this apparatus is located on a tabletop. On each test day, each hand/hindpaw is tested twice in an alternating order (i.e., left, right, 1 min wait, left, right), and these scores are averaged to yield a single score for analysis. This average hand/hindpaw withdrawal latency (in seconds) is the thermal nociception score for each subject (i.e., lower scores indicate hyperalgesia). **Von Frey Method:** To assess mechanical sensitivity, each hand (human) or hindpaw (NHP) is stimulated using an electronic von Frey apparatus (Stoelting, Wood Dale, IL). Subjects are first acclimated for 10 minutes to the testing room/apparatus, which for NHP consists of a chair and a mesh stand floor suspended 30 cm from the ground. For humans, this apparatus is located on a tabletop. The force-measuring filament is applied perpendicularly to the plantar surface of the hand/hindpaw until it buckles for 2 seconds, and a sharp

withdrawal of the stimulated hand/hind paw within the 2 seconds indicates a positive response. On each test day, each hand/hindpaw is tested twice in an alternating order (i.e., left, right, 1 min wait, left, right), and these scores are averaged to yield a single score for analysis. This average hand/hindpaw withdrawal force (in grams) is the mechanical nociception score for each subject (i.e., lower scores indicate hyperalgesia). **Western Analysis:** Brain punches will be processed for Westerns as previously published.[57] For phosphoprotein detection and normalization, corresponding total proteins (e.g., GR) are labeled following phosphoprotein detection (e.g., pGR) via stripping and re-probing techniques. Changes in total protein will be normalized to tubulin levels. Excess homogenates and alternative brain regions will be stored at -80ºC for future examination and available to CARC and outside investigators. **Electrophysiological Analysis:** Following necropsy, brain tissue will undergo a series of steps designed to preserve and extend normal physiology of primate brain tissue. First, tissue will be dissected and transported in ice-cold oxygenated (95%O2–5% CO2) artificial cerebrospinal fluid (ACSF, concentrations in mM: 130 NaCl, 3.5 KCl, 2 CaCl2, 1.25 NaH2PO4, 1.5 MgSO4·7 H2O, 24 NaHCO3, 10 glucose) to electrophysiology lab where slices 300 µm in thickness will be prepared using a vibrating microtome (Leica Microsystems). Coronal tissue slices containing the lateral PFC and anterior cingulate cortex will be collected and placed in in a NMDG-HEPES based ACSF solution (92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 CaCl2·2H2O, and 10 MgSO4·7H2O) at physiological temperature (~37°C) for 12 minutes used to reduce excessive excitotoxicity in the tissue. Then, slices will be transferred to a ultraviolet light treated holding solution (92 NaCl, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 2 CaCl2, 2 MgSO4·7 H2O, 5 Na-ascorbate, 3 Na-pyruvate) replaced every two hours. Whole-cell patch clamp recordings will be made under DIC visualization and pyramidal neurons will be targeted based on their characteristic shape. In each recording day, two recording conditions will be used: 1.) for intrinsic properties under current clamp conditions, recording electrodes will be filled with K+gluconate internal solution (in mM: 126 K-gluconate, 10 HEPES, 0.3 EGTA, 4 KCl, 0.3 Na2-GTP,4 Mg-ATP, 10 Na2-phosphocreatine, 0.4% Biocytin), 2.) to measure excitatory postsynaptic currents (EPSCs), a Cs-Gluconate solution (117 mM Cs + gluconate, 20 mM Hepes, 0.4 mM EGTA, 5 mM tetraethylammonium, 2 mM MgCl2, 4 mM Mg-ATP, and 0.3 mM Na2 GTP will be isolated with 25 µM picrotoxin and evoked by local fiber stimulation with bipolar nichrome electrodes (5–30 pA with a 100–150 µs duration, 0.0167 Hz). For current clamp experiments, passive (RMP, input resistance, capacitance, membrane tau), active (voltage sag, rheobase, afterdepolarization) and firing (action potential peak, half-width, threshold, input-output) properties cells will be measured. In each recording, whole cell stimulus parameters will be repeated following bath application of 20 and 50 mM ethanol and washout to determine how acute alcohol effects intrinsic and synaptic physiology. **Spine Density Analysis:** Following recordings, slices will be fixed in 4% paraformaldehyde and processed for dendritic spine visualization. Recorded neurons will be visualized using Alexa 647 conjugated streptavidin. In addition to recorded neurons, several non-recorded neurons will be filled post fixation with Lucifer yellow using sharp electrodes as described previously.[58] A confocal microscope (Olympus FV1000) will be used at low (20x) and high (60x) objectives to image neuronal morphology and dendritic spine density in 3 dimensions. Images will be analyzed by researchers blind to condition using Neurolucida 360 software. Dendritic arborization and spines will be counted and classified according to their characteristic morphology (thin, stubby, mushroom). Comparisons will be across conditions and morphological measurements from recorded neurons will be correlated with their electrophysiological properties (e.g. spine count and eEPSC) to determine functional relevance of structural properties across groups.[59] **Statistical Analysis & Power Analysis: Sample size justification for Aims 1 and 2:** For human subjects, the sample size justification for Aim 1 is based on the comparison of the cognitive assessment, MoCA, between patients with alcohol use disorder (AUDIT >= 16) and those without alcohol use disorder (AUDIT < 8). In the NOAH PLWH cohort, 62 subjects have an AUDIT score of 16 or higher, with 218 less than 8. Based on preliminary data, 15 subjects with an AUDIT score of 16 or higher have received a cognitive assessment with an average score of 18 and standard deviation of 4.58. In the AUDIT < 8 group, 94 subjects have received the assessment and have exhibited an average score of 20.8 with a standard deviation of 4.69. We also plan to enroll 250 seronegative subjects. Assuming 25% less subjects with AUDIT scores of 16 or higher in the HIV- cohort, we expect to see about 33 subjects with AUD in the HIV- cohort. Based on preliminary estimates in the NOAH cohort and results presented in Likhitsathian et al (2012),[60] we expect HIV to reduce MoCA by 4 points and AUD to reduce MoCA by about 2.7 points. Along with a standard deviation of 4.5, this leads to power to detect an AUD effect of about 80%, with power close to 100% for the HIV effect. A total of 40 NHPs will be studied, with 10 subjects in each of the four groups based on alcohol exposure and SIV status (2x2 design), all fed a WD. According to pilot data, the mean difference in interaction time between the CBA group and the VEH group is about 60 seconds. The SD for interaction times is 38 seconds. This leads to 90% power to detect a difference between the CBA and VEH group. The effect of SIV/ART is unknown, but

the fact that interaction time is non-negative dictates that either the effect is smaller for SIV/ART than for CBA or there is an interaction between SIV/ART and CBA. Assuming the main effect of SIV/ART reduces interaction time by 48 seconds or more, the power to detect a difference will be at least 80%. **Sample size justification for Aim 3:** GR in the PFC is the primary outcome for Aim 3. Based on pilot data, we expect CBA to increase GR levels by 59%, with SD for GR levels at 30%. The current design provides at least 80% power to detect increases due to SIV/ART if GR levels are increased by at least 40%. **Statistical Considerations:** For the human studies, there are 365 PLWH in the NOAH cohort, and a cohort of 250 HIV- subjects will be enrolled. For the NHP studies, we will have a total of 40 NHPs. There are 10 NHP subjects in each of the four groups based on alcohol use and SIV status. The primary outcome in humans for Aims 1 and 2 is cognitive impairment as measured by the MoCA score. This will be analyzed as a continuous outcome with a two-way ANOVA. For the effect of alcohol use, primary interest is in the comparison between subjects with AUDIT score above 15 and subjects with a score below 8. However, subjects with AUDIT between 8 and 15 will be included in the analysis since doing so results in a more powerful test for both the effect of alcohol and the effect of HIV/ART. The three groups, AUDIT less than 8, AUDIT between 8 and 15, and AUDIT greater than 15, will be treated as categorical variables. The same two-way ANOVA strategy will be used for the results of the Hargreaves and von Frey assays in humans and NHPs in addition to the tail dip assay in NHPs. For Aim 3, a two-way ANOVA will be used to determine the differences in GR activity in the NHP subjects. Longitudinal data in humans will be modeled using linear mixed models, with PBMC GR signaling as the response variable. Covariates will include HIV status and AUDIT score. Other important covariates (e.g., dietary factors) will be included as needed to improve estimation of HIV and alcohol effects. **Integration of Data with Other RCs:** Data will not only provide neurobiological insight into how cognitive deficits and pain-related behaviors are manifest in PLWH who drink, but will be integrated with data from the other RCs. Specifically, analysis in the context of environmental and psychosocial stress factors (RC1) will establish associations and link to causal factors in the environment to frequency of neurological deficits and potential identification of peripheral (PBMC) glucocorticoid signaling as a biomarker of psychosocial stress. Integration with RC2 will inform on the link between metabolic dyshomeostasis and neurocognitive comorbidities by analyzing the frequency of low scores on MOCA with markers of metabolic dyshomeostasis. Indices of immunosenescence and senescent cell tissue presence (RC4) will be integrated into the interpretation of findings and correlated with measures of GR signaling and possible peripheral glucocorticoid resistance. **Rigor and Reproducibility:** As described throughout this application we have given careful consideration to the strengths and weaknesses of published research and preliminary data that form the basis of this proposal. All experiments will be performed with independent biological replicates. Whenever possible, experimental groups will be blinded to the investigator. The experimental study design and most of the procedures that we are proposing are performed routinely and will be performed following published standard operating procedures in order to produce robust and unbiased results. Reagents, antibodies and commercial kits used will be purchased from reputable commercial vendors (Authentication of Key Biologicals). All experiments and assays will use appropriate positive and negative controls. All personnel will follow institutional training that covers use of human subjects and humane care and use of animals. **Sex as a Biological Variable:** Our preliminary preclinical studies supported by CARC pilot funding in the previous cycle examined cognitive function (NOR task, **Figure 6**) and neuroadaptations (GR levels and phosphorylation, **Figure 8**) in female NHPs. In addition, our NOAH clinical cohort includes close to 30% adult female subjects and analyses were conducted to dissect the role of sex and gender on the outcomes examined. Our preliminary results analyzing cognitive function from this cohort (MoCA, **Figure 5**) do not identify significant or salient sex-specific differences, nor did we find that OVX aggravated any of the NHP outcomes examined. This observation may be limited by the number of subjects studied (both human and NHP samples). The proposed clinical studies will include both males and females and data will be analyzed to dissect the potential role of sex. However, the NHP studies will be conducted in male rhesus macaques. One of the major limitations we encountered during the past cycle was the scarcity in adult female macaques for these studies due to their value for breeding and more urgently for Zika studies. Nevertheless, we do not discard the possibility of future NHP studies using both males and females if our clinical data reveals sex differences in the proposed study outcomes.

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
Other Requested Information	RC3_Other_Requested_Information_NOAH_CARC_2019.pdf

Other Requested Information RC3 LSUHSC CARC

Details of the New Orleans Alcohol Use in HIV (NOAH) Study are included in the Overall Component.

Human Subject Studies

Study#	Study Title	Clinical Trial?
The form does not have any study records		

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

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Specific Aims:

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LSU Health NEW ORLEANS
School of Medicine
Department of Cell Biology and Anatomy

Department of Cell Biology and Anatomy
School of Medicine
LSU Health Sciences Center
[redacted]
New Orleans, LA, 70112

April 9, 2019

Dear Dr. Edwards,

Based on our intriguing preliminary findings supported by your CARC pilot grant, I'm very happy to continue to work with you and [redacted] on the proposed renewal experiments. As you know, I have focused my career on examinations of cortical plasticity in neural disease states, including work on our collaborative NIAAA R01 grant with CARC Director Dr. Patricia Molina. The proposed *ex vivo* recordings from macaque frontal cortex extend on our data collected from the striatum, and will provide valuable synaptic information pointing to functional neuroadaptations following alcohol and SIV exposure with the background of a Westernized diet. These data will also tie in well the behavioral and biochemical endpoints and make RC3 truly integrative in nature as well as translational to inform ongoing work in the NOAH human cohort. Our publication and research history demonstrates the strong partnership between our labs and departments, and I look forward to leveraging this success for these new aims.

[redacted]

Resource Sharing Plans LSUHSC CARC Overall

1. Data Sharing Plan

The proposed research includes both human and animal studies.

Requests for access to existing clinical data or biological samples from these studies will be considered by the Intramural Center Committee (ICC). A formal request delineating the data-sharing agreement will include the following: a commitment to using the data only for research purposes and not to identify any individual subject. For both animal and human data sharing, (a) assurance that data will be used in accordance with the NIH Public Access Policy regarding submission to peer-reviewed journals and submission of the published manuscript to the digital archive of PubMed Central no later than 12 months after publication; (b) assurance that the original values within the data set will not be altered in any way; (c) assurance that the data will be secure using appropriate computer technology and that the data will not be distributed to a third party; (d) assurance that the data will not be used for commercial purposes or to raise money by an individual or affiliated organization; and (e) acknowledgment that the Louisiana State University Health Sciences Center (LSUHSC) Comprehensive Alcohol-HIV/AIDS Research Center (CARC) grant is the data resource in scientific publications; will be requested.

Nonhuman primate studies will be performed over a 5-year period. Serial tissue samples will be obtained from these animals and multiple tissues will be available at the time of necropsy. The comprehensive data set will include animal health records and laboratory data from blood, cell, tissue, and biological fluid samples collected throughout the course of the study. Thus, spin-off pilot projects using nonhuman primate tissues or data obtained but not used for the main research components will be feasible. Access to this data set will be available to intra- and extramural investigators interested in expanding the focus of research. Rhesus macaques are a valuable resource and few research facilities and investigators are able to conduct research with this model. Therefore, requests for access to biological samples obtained from our animals will be accepted from researchers not affiliated with this CARC. The ICC will evaluate these requests based on the following guidelines: (1) executing the request does not interfere with the primary studies presented in this application; (2) IACUC approval is obtained; (3) the collected biological sample is scientifically justified to have potential benefit to understanding the impact of alcohol on SIV disease prevention, progression or treatment; (4) costs of obtaining the biological sample are agreed upon based on relatedness to the current proposal. Necropsy samples are available from study animals without IACUC approval and can be provided upon request. Because of cost and storage limitations, tissues not directly needed for existing projects are not collected. In order to maximize the possibilities of providing necessary tissues to other investigators, advanced notice will be sent to those investigators that have contacted the LSUHSC CARC requesting access to tissues when the time of necropsy is determined. Processing, shipping, and handling of the requested samples will be covered by the requesting individual. Biological transfer requests will be approved based on submission of a material transfer agreement, to be executed through the Office of Research Services of the LSUHSC. Priority will be given to those projects that have greater promise to develop new areas of investigation or to synergize with existing research interests of the CARC as evaluated by the ICC.

Researchers requesting access to data or biological specimens will obtain access upon the signing of the data-sharing agreement by the requesting investigator and an authorized representative of the recipient institution. All requests will be processed by the LSUHSC Office of Research Services in accordance with LSUHSC's policy on material and data transfer.

The CARC will comply with the new data-sharing initiative as created by the National Institute on Alcohol Abuse and Alcoholism (NIAAA). In order to fulfill these requirements, the NIAAA data archive (NDA) data-sharing agreement will be completed and submitted within 6 months of the notice of award issue date as well as reporting expected measures from all CARC human subjects studies. All CARC research subjects will be notified of the intent to broadly share de-identified data resulting from their participation and informed consent will be obtained after this notification. In addition, all CARC studies will collect the following information to create a Global Unique Identifier (GUID): sex, first name, last name, middle name, date of birth, and city/municipality of birth as it appears on the birth certificate. The data management unit, within the Administrative Core, will work to harmonize all CARC human subjects data to match the data definitions and best practices of the NDA. After the data management unit has created a GUID for each CARC research subject, the de-identified data will be submitted to the NDA twice a year, on or before the due dates. Any quality control issues will be addressed by the data management team. Prior to publication or presentation, the Administrative Core will create an NDA study record and therefore create a digital object identifier, linking the subject-level data to the result.

2. Sharing Model Organisms
This CARC proposal does not anticipate the development of model organisms.

3. Genome Wide Association Studies (GWAS)
This proposal does not include a GWAS.

Authentication of Key Biological and/or Chemical Resources LSUHSC CARC Overall

Biospecimen Collection and Processing. Biospecimens from humans and macaques will be processed in our BSL-2 facility within two hours of receipt of the sample. All techniques for processing blood and tissues are routine in our lab. Processed aliquots will be stored in pre-labeled, barcoded containers, and specimen data will be entered into our electronic laboratory information management system (LIMS). Relevant clinical data will be entered into the LIMS in association with the appropriate samples under the direction of the laboratory manager.

Chemicals. All acquired compounds and reagents will be purchased from commercial sources that have authenticated the reagents for both identity and purity using standard techniques and methods for the characterization of small molecules according to the guidelines of the American Chemical Society. This information will be included in peer-reviewed publications, along with details on commercial sources for precursors and any necessary purification, handling, and storage of reagents and products (e.g., under an inert atmosphere). 5-bromo-2'-deoxyuridine (BrdU), FITC-dextran (3kD), and Texas Red-dextran (40kD) will be purchased from ThermoFisher Scientific for *in vivo* macaque studies.

Antibodies. Antibodies for flow cytometry and Western blotting will be obtained from commercial manufacturers providing hybridoma clone identification for monoclonal antibodies, lot number, and appropriate references. The specificities of the antibodies employed have been validated by the manufacturer, and compensation setup and tracking bead controls will be included in each flow cytometry experiment. All Western blotting procedures will include positive and negative controls for proteins of interest. We will monitor the Antibody Registry database (http://antibodyregistry.org/) to be aware of any issues observed by other investigators with antibodies we propose to use. All antibodies will be purchased from the same lot. We propose multiple fluorescence-activated cell sorting (FACS) panels in this application. Panel 1 will include CD3-V500 (clone SP34-2), CD4-Qdot655 (clone 53.5), CD8-Qdot605 (clone 3B5), CD28-BV421 (clone CD28.2), CD38-PE (clone HB-7), CD45-AF488 (clone H130), CD45RA-APC (clone HI100), CD95-PE-Cy5.5 (clone DX2), CD197-PE-DAZZLE594 (clone G043H7), CD279-PE-Cy7 (clone EH12.2H7), HLA-DR-AF700 (clone L243), CD14-PerCP-Cy5.5 (clone M5E2), CD69-BV711 (clone FN50), CD25-BV510 (clone BC96), Live Dead-eFluor780, and BrdU-Qdot585 (clone 3D4). Panel 2 will have the following substitutions for the specified fluorophores: EdU-AF488; IFNγ-APC (clone 25723.11); IL-17-BV421 (clone BL168); IL-2-PerCP-Cy5.5 (clone MQ1-17H12); IL-22-PE-Cy7 (clone 2G12A41); TNFα-PE-Dazzle594 (clone MAb11). Data will undergo quality control measures and be certified by the FACS lab technical director and directors of the Experimental and Analytical Core before entry into the database.

ELISA, Multiplex, and Radio Immunoassays (RIA). We propose to measure intestinal fatty acid binding protein (iFABP), soluble CD14 (sCD14), high-mobility group box 1 (HMGB1), insulin, C-peptide, adiponectin, glucagon and alpha-1 antitrypsin (A1AT) by ELISA. Additionally, we will measure IL-1β, IL-2, IL-10, IL-17, and IFN-γ by multiplex assay. All assays will be purchased in bulk from commercial vendors to ensure the same lot will be used for each assay. Each assay will include control plasma and low-, medium-, and high-protein spikes corresponding to values at 15, 50, and 85% of the standard curve concentrations to control for plate-to-plate variation and verify recovery from the matrix. Data will undergo quality control measures as specified by the manufacturers and certified by the lab supervisor and directors of the Experimental and Analytical Core before being entered into the database.

qPCR and RT-qPCR. Quantitative PCR (qPCR) for telomere length and RT-qPCR for SIV and HIV viral load will be performed on PBMCs and plasma respectively. All primers, probes, and standards have been designed using Integrated DNA Technologies PrimerQuest tool and OligoAnalyzer and tested for quality assurance by the manufacturer. For telomere qPCR, two housekeeping single copy genes will be amplified: ribosomal protein lateral stalk subunit P0 (RPLP0) and beta-2 microglobulin (B2M). DNA from HEK293 cells will be purchased from Sigma-Aldrich, and DNA isolated from pooled PBMC samples will be generated in our laboratory. These will serve as endogenous controls to allow for assessment of plate-to-plate variation. RT-qPCR and qPCR assays are capable of detecting a single copy of SIV/HIV RNA or DNA (within limits of Poisson distribution), and with validated reproducible detection of five copies when samples are analyzed in duplicate. HIV/SIV values are normalized to a single-copy gene (for DNA assays), a validated RNA transcript (RPS13 for RNA assays), or volume of fluid for virion quantification in plasma and secretions. The HIV **assay** has been validated through comparisons with clinical data. Aliquots of HIV and SIV positive and plasma samples, as well as absolute DNA and RNA standards have been preserved and are included in every assay to validate the accuracy of standard curves used for quantification of virus in samples. Criteria for confirmation of acceptable standard curves, results from standards, and negative controls are included in our protocols. Raw data is reviewed by the lab supervisor and directors of the Experimental and Analytical Core before entry into the database.