Notice of Award

SPECIALIZED RESEARCH CENTER
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 12/06/2017

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Grant Number: 1P50AA026117-01
FAIN: P50AA026117

Principal Investigator(s):
JEFFREY L WEINER, PHD

Project Title: Wake Forest Translational Alcohol Research Center (WF-TARC)

[redacted]
Winston-Salem, NC 271570001

Award e-mailed to: nihawards@wakehealth.edu

Period Of Performance:
Budget Period: 12/10/2017 – 11/30/2018
Project Period: 12/10/2017 – 11/30/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,605,748 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to WAKE FOREST UNIVERSITY HEALTH SCIENCES 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 P50AA026117. 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,

Judy Fox
Grants Management Officer
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Additional information follows

SECTION I – AWARD DATA – 1P50AA026117-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$514,316
Fringe Benefits	$107,604
Personnel Costs (Subtotal)	$621,920
Consultant Services	$1,350
Equipment	$7,200
Materials & Supplies	$198,928
Travel	$10,350
Other	$182,635
Subawards/Consortium/Contractual Costs	$14,643
Publication Costs	$900
ADP/Computer Services	$594
Federal Direct Costs	$1,038,520
Federal F&A Costs	$567,228
Approved Budget	$1,605,748
Total Amount of Federal Funds Obligated (Federal Share)	$1,605,748
TOTAL FEDERAL AWARD AMOUNT	$1,605,748
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,605,748

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$1,605,748	$1,605,748
2	$1,607,584	$1,607,584
3	$1,602,585	$1,602,585
4	$1,602,773	$1,602,773
5	$1,602,858	$1,602,858

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:	1223849199A1
Document Number:	PAA026117A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019	2020	2021	2022
AA	8470434	$1,605,748	$1,607,584	$1,602,585	$1,602,773	$1,602,858

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

NIH Administrative Data:
PCC: AN Q / **OC**: 414A / **Released**: [redacted] 11/30/2017
Award Processed: 12/06/2017 12:02:00 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1P50AA026117-01

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

SECTION III – TERMS AND CONDITIONS – 1P50AA026117-01

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

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

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

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

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

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) P50AA026117. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

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

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

This award provides support for one or more clinical trials. By law (Title VIII, Section 801 of Public Law 110-85), the "responsible party" must register "applicable clinical trials" on the ClinicalTrials.gov Protocol Registration System Information Website. NIH encourages registration of all trials whether required under the law or not. For more information, see http://grants.nih.gov/ClinicalTrials_fdaaa/

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that

reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AA Special Terms and Conditions – 1P50AA026117-01

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

In accordance with NIH Guide Notice NOT-OD-17-124 (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-124), all legislative mandates that were in effect in FY 2017 (see NOT-OD-17-075) remain in effect, including the salary limitation set at Executive Level II of the Federal Pay Scale as described in NIH Guide Notice NOT-OD-17-087. None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the salary cap. Therefore, this award and/or future years are adjusted accordingly, if applicable.

This grant has been identified as requiring a Data & Safety Monitoring Plan (DSMP) in accordance with the NIAAA Data and Safety Monitoring Guidelines at http://www.niaaa.nih.gov/research/guidelines-and-resources/data-and-safety-monitoring-guidelines. The NIAAA Program Official named below has approved the DSMP as submitted by the grant recipient.

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: Lauren Early
Email: earlyle@mail.nih.gov **Phone**: 301-443-2434

Program Official: Qi-ying Liu
Email: liuqiy@mail.nih.gov **Phone**: 301-443-2678 **Fax**: 301-443-1650

SPREADSHEET SUMMARY
GRANT NUMBER: 1P50AA026117-01

INSTITUTION: WAKE FOREST UNIVERSITY HEALTH SCIENCES

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$514,316	$535,151	$541,205	$541,205	$537,965
Fringe Benefits	$107,604	$113,551	$115,314	$115,314	$114,310
Personnel Costs (Subtotal)	$621,920	$648,702	$656,519	$656,519	$652,275
Consultant Services	$1,350	$13,050	$1,350	$11,250	$5,850
Equipment	$7,200				
Materials & Supplies	$198,928	$190,518	$168,797	$164,712	$177,047
Travel	$10,350	$13,950	$15,750	$15,750	$15,750
Other	$182,635	$155,414	$175,145	$173,074	$165,015
Subawards/Consortium/Contractual Costs	$14,643	$14,643	$14,643	$14,643	$14,643

Publication Costs	$900	$1,800	$6,323	$2,700	$8,123
ADP/Computer Services	$594	$594	$594	$594	$594
TOTAL FEDERAL DC	$1,038,520	$1,038,671	$1,039,121	$1,039,242	$1,039,297
TOTAL FEDERAL F&A	$567,228	$568,913	$563,464	$563,531	$563,561
TOTAL COST	$1,605,748	$1,607,584	$1,602,585	$1,602,773	$1,602,858

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	55%	55%	55%	55%	55%
F&A Cost Base 1	$1,031,323	$1,034,387	$1,024,480	$1,024,601	$1,024,656
F&A Costs 1	$567,228	$568,913	$563,464	$563,531	$563,561

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 9377279070000

Legal Name*: Wake Forest University Health Sciences
Department: Physiology_Pharmacology
Division:
Street1*: [redacted]
Street2:
City*: Winston-Salem
County:
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27157-0001

Person to be contacted on matters involving this application

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

[redacted]

Position/Title: OSP Administrator I
Street1*: Wake Forest University Health Sciences
Street2: Medical Center Boulevard
City*: Winston-Salem
County: Forsyth
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27157-0001
Phone Number*: [redacted] Fax Number: [redacted] Email: nihawards@wakehealth.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

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

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 2: Mechanisms underlying vulnerability to ethanol self-administration: behavioral and brain imaging studies in group-housed monkeys

12. PROPOSED PROJECT

Start Date*	Ending Date*
12/01/2017	11/30/2022

OMB Number: 4040-0010
Expiration Date: 06/30/2018

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: Wake Forest University Health Sciences
Duns Number: 9377279070000
Street1*:
Street2:
City*: Winston-Salem
County:
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27157-0001
Project/Performance Site Congressional District*: NC-005

Additional Location(s) File Name:

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00248

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*	Proj2_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Proj2_literature_cited.pdf
10.Facilities & Other Resources	Proj2_res_facilities new_1.pdf
11.Equipment	Proj2_ResourcesEquip_full_draft.pdf

PROJECT SUMMARY

Alcohol use disorder (AUD) persists as a costly public health problem that lacks widely effective medications and strategies for prevention. The overarching scientific premise of this Project, like the others of the Wake Forest Translational Alcohol Research Center (WF-TARC), is that the neural substrates that contribute to vulnerability and resilience to AUD are not fully understood. Studies using nonhuman primate (NHP) subjects have specific advantages that make them a critical part of a comprehensive, translational approach to addressing this topic, including the possibility of experimental control not possible in human subjects and a greater similarity to humans' neurobiology compared to rodents. Group-housed monkeys form linear social hierarchies; social rank has been shown to influence sensitivity to abuse drugs, with subordinates showing vulnerability to the abuse-related effects of stimulants and ethanol (EtOH). Project 2 of the WF-TARC will exploit this differential sensitivity across social ranks to determine the behavioral and brain mechanisms that underlie vulnerability to develop AUD. Behavioral studies will characterize rank-related differences in induction of EtOH drinking, EtOH consumption over one year of 22 hours-per-day access and EtOH seeking behavior during abstinence using an extremely well-characterized NHP EtOH self-administration model of long-term drinking in humans. We will also determine whether dominant and subordinate monkeys differ in sensitivity to chronic treatment potential medications for AUD. In parallel to these experiments, brain imaging studies using magnetic resonance imaging will characterize the structural and functional differences between dominants and subordinates, and determine the specific changes that occur in grey and white matter integrity, cerebral blood flow and functional connectivity during long-term EtOH drinking and subsequent abstinence. Importantly, these NHP studies occupy a critical position in the translational structure of the WF-TARC, supporting forward and backwards translation to inform and extend findings in rodent and human projects. NHP imaging studies will focus on the same brain regions and nodes that will be imaged in human subjects and studied and manipulated in rodents. Secondary analyses on imaging data will expand this focus to the entire brain. Taken together, the results of the studies in this Project, particularly in combination with data generated in other components of the WF-TARC, will provide a comprehensive account of brain differences between populations that are resistant versus vulnerable to AUD. This knowledge will ultimately help practitioners direct preventive efforts to groups who will most benefit from them, and will identify new targets for more effective medications targeted to the most vulnerable populations.

PROJECT 2

RESOURCES: FACILITIES AND OTHER RESOURCES

Wake Forest School of Medicine facilities and resources relevant to the proposed research fall into three categories:

1) The non-human primate ethanol self-administration laboratory of Dr. Paul Czoty (Project PI) and [redacted]
2) The Radiology Informatics and Image Processing Laboratory (RIIPL): directed by [redacted]
3) Resources available through the Wake Forest Translational Imaging Program

1. Non-human primate ethanol self-administration laboratory of Drs. Paul Czoty and [redacted]

Laboratory: The proposed behavioral research will be conducted in the Department of Physiology and Pharmacology in the Wake Forest School of Medicine, a component of Wake Forest University Health Sciences (WFUHS). Animal housing and laboratory facilities specifically designed to conduct the experiments are located in [redacted]). In short, everything needed to conduct these studies is in place and functioning in the laboratory. [redacted], there are two 400ft^2 wet laboratories [redacted]. They contain chemical hoods, sinks, counters, scales, clinical centrifuges, freezers and two flammables cabinets. In addition, they house materials and equipment for cleaning and autoclaving instruments and glassware, preparing drugs (Mettler balances, a pH meter, hot/stir plate and associated supplies), analyzing data, preparing enrichment items and repairing equipment. Refrigerators and freezers are also located in this space. One of these laboratories also houses all equipment necessary for analyzing blood samples for ethanol content, including a gas chromatograph (Agilent 7890A GC system with G1888 Network Headspace Autosampler Santa Clara, CA) supplied with a flame ionization detector and Agilent ChemStation integrator.

The following animal resources are currently in use for an ongoing chronic ethanol self-administration study in rhesus macaques, and will be available for use for the proposed research. Monkeys are housed individually within a 6.2 sq. ft. stainless steel cage [redacted] (Allentown Caging Equipment Co., Inc.) in a temperature- and humidity-controlled vivarium (see below). Attached to one wall of each animal's home cage is an operant panel that allows access to all fluid and food requirements and controls all experimental events. The panels were constructed with two drinking spouts, a set of 3 lights positioned above each spout, one finger-poke response system and one food cup connected to a pellet feeder. A centrally located opening contains a dowel that must be pulled to allow access to all fluids and food. Each drinking panel is connected to individual ethanol and water reservoirs which sit atop two separate balances. The balances rest on a rack system adjacent to the home cage and continuously monitor weight displacement that is subsequently converted to volume displacement to determine fluid consumption. Events are controlled and data are recorded with a National Instruments interface connected to a MacIntosh G4 computer.

Animal Housing: The monkeys that will serve as subjects in the proposed studies will reside in a state-of-the-art vivarium [redacted] which has space [redacted] under the administration of the Animal Resources Program (ARP). ARP also provides necessary space for quarantine of newly purchased animals and complete veterinary service. The animal facilities have 24-hour security. The vivarium includes a surgical suite with three operating rooms, an animal preparation room and a surgeon and instrument preparation room, all adjacent to the operating rooms. The necropsy suite, located adjacent to the surgery suite, consists of an anteroom for personnel preparation and a primary necropsy room with a stainless steel necropsy bench, irrigation and downdraft air handling system. An adjacent stainless steel countertop contains sinks and space for instrument and equipment placement. A cold room is attached to the suite on one side for disposition of animal remains and a pathology suite on the opposite side for pathology procedures. The Wake Forest School of Medicine is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Imaging resources. [redacted] has 1 Dispomed Optimax anesthesia machine equipped with a Hallowell ventilator and 2 Narkomed 2B anesthesia machines equipped with ventilators for providing gaseous anesthesia. These machines are equipped with Capnomac monitors for monitoring respiration rate CO_2 levels and other physiological signs during imaging sessions. A Nonin pulseoximeter is used to monitor heart rate

and oxygen saturation levels. He also has a dedicated 8-channel flexible phased array coil built by [redacted] that was designed to accommodate the monkey skull. He also has a dedicated RF coil (Litzcage, Doty Scientific, Columbia, SC) for use in non-human primates.

Although the proposed research does not involve terminal studies, we will have at our disposal a wealth of resources associated with the Monkey Alcohol Tissue Research Resource (www.matrr.com), a NIAAA-funded resource on which [redacted] is a Co-I and lead investigator at WFUHS. The primary goal of this site is to build the resources of a tissue bank and associated bioinformatics to analyze and distribute appropriate tissue samples to the alcohol research community. Among the MATRR resources on [redacted] are:

- one Leica cryostat which is available for cryo-sectioning brains and which is under a maintenance agreement.
- an Image Analysis suite that contains the AIS/C Image analysis system; Coolsnap digital camera and lightbox, and a Nikon Digital Sight DS-5i1 digital image capture system and NIS-Elements Software that can be connected to the Leitz Orthoplan microscope or the Nikon SMZ 1500 Stereo Dissecting Zoom microscope for cytoarchitectonic analyses. The SMZ 1500 is also equipped with Nikon Intenslight C-HGFI for fluorescence microscopy. The suite also contains equipment for anatomical tracing procedures and a high resolution HP laser printer. One room houses a Leica CM3050S cryostat for brain cryosectioning and related equipment used for processing blood and tissue samples, drug preparation, preliminary analysis of behavioral data, GC analysis for blood ethanol and acetaldehyde, and -80°C freezer space for brain tissue.
- the autoradiography core located within the laboratory space described above. It contains equipment for *in vitro* receptor autoradiography, *in situ* hybridization, phosphorimaging, immunocytochemistry, tissue homogenate receptor binding and additional -80°C freezers for storage.
- Two -80°C freezers located in adjacent space.
- A darkroom (150 sq. ft.) that is shared by departmental faculty. This room contains equipment for development of x-rays and standard photographic equipment.

Office: Office space for Drs. Czoty and [redacted] and laboratory technicians and fellows/graduate students is located [redacted]

Computer: Several computers for word processing and routine data analysis are available, including a Dell Optiplex Intel Core Quad CPU, 3.0 GHz and an Apple MacBook Pro laptop. An MCID image analysis system is run on a Pentium IV, 1.6 GHz computer and is located [redacted] 2 Dell pc workstations and 2 Wacom LCT monitors are available for additional image analysis. [redacted] has 8 network drives with a total of 4 TB storage space for archival of imaging data. Ample storage is available in the laboratory and on servers maintained by the Department and the Wake Forest School of Medicine.

2. Radiology Informatics and Image Processing Laboratory (RIIPL): Director, Dr. Christopher Whitlow

A. Laboratory. RIIPL at WFUHS encompasses approximately 3000 square feet of space in the Department of Radiology specifically for conducting image processing related research. The space contains a large conference room for laboratory meetings, data presentations, and journal club meetings, as well as a computer lab containing Linux and Solaris machines for general use and data analysis.

B. Office. All neuroradiology fellows, post-docs, and graduate students associated with the RIIPL have personal cubicle space. All faculty have at least 100 square feet of office space. Secretarial support is available through the Department of Radiology.

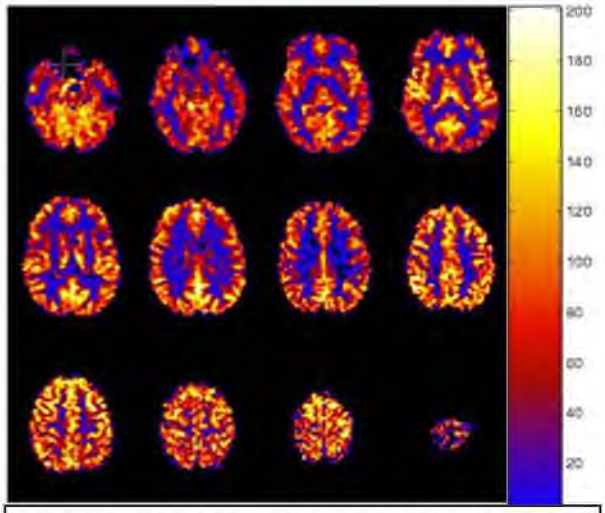


Figure 1. ASL CBF map in a young normal subject.

C. RIIPL computer infrastructure. The RIIPL maintains a rack-configured cluster dedicated to distributed image processing consisting of 13 Linux computers providing 71 compute nodes scheduled through the SUN Grid Engine. Computing power includes five 3 GHz dual core units with 4GB RAM, five 3.3GHz dual core units

with 16GB RAM, one 2.8 GHz dual 6-core unit with 32GB RAM, one 3.5GHz dual 6-core unit with 96GB RAM, and one 3.5GHz dual 6-core unit with 192GB RAM that also contains four C2070 Nvidia Tesla GPUs. Nine terabytes of network-attached storage are available and connected over a gigabit Ethernet backbone. All computers are configured to run Matlab via a floating license and are used for functional image processing and analysis. These computers can communicate directly with the Department of Radiology DICOM digital archive, as well as with the clinical and research MRI and CT scanners. All computers are password protected and secured behind the University firewall, and data storage is maintained and backed up by WFUHS Information Services group. The RIIPL imaging infrastructure facilitates the processing of large amounts of neuroimaging data and provides an interface to SPM/FSL/XNAT.

D. Additional data processing infrastructure. We also have a TeraRecon server (AquariusNET and iNtuition software) with 10TB of high performance RAID storage, TeraRecon APS server, Siemens MMWP workstation, Mac servers running DCM4CHEE (research DICOM servers), virtual servers Matlab, DCM4CHEE, and XNAT PC and Mac workstations.

E. XNAT and data archival. XNAT is an open source imaging informatics platform designed to facilitate management and exploration of medical imaging and related data. XNAT includes a DICOM workflow, a secure database backend, and a rich web-based user interface. XNAT's web services interface enables external applications and institutional investigators to easily access XNAT-hosted data. The RIIPL currently supports and administers XNAT databases for investigators that perform brain imaging studies, including access to the Diabetes Heart Study (DHS-Mind) of over 600 participants, and the African American DHS-Mind study of ~600 participants. The XNAT databases are project specific, and allow storage, retrieval, and complex queries of imaging and metadata. The images, databases and other critical system data are maintained by Information Services at WFUHS.

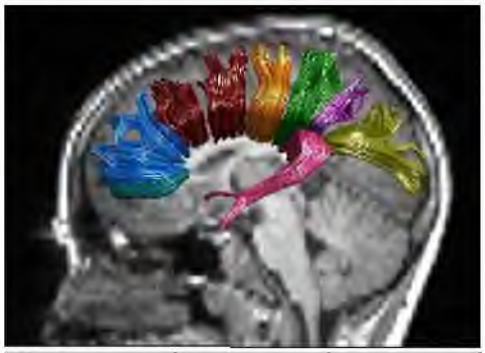


Figure 2. Automated Fiber Quantification. Regional tracts of corpus callosum overlaid on structural T1 weighted image.

F. RIIPL brain MRI (structural, diffusion, perfusion, network) processing pipelines. RIIPL investigators (Whitlow and colleagues) at WFUHS have been at the forefront of functional imaging processing methods, implementing a fully automated processing pipeline in 2001, including features such as distributed grid processing, automated error recovery, and data provenance. This has enabled WFUHS to become the leading institution in the world in clinical ASL, with seamless translation of image acquisition, automated post-processing, and insertion into the Picture Archiving and Display System (PACS), with over 25000 clinical ASL MRI studies performed over the last 3 years. Our implementation has shown excellent reproducibility in normals (**Figure 1**), and sensitivity to a variety of disease states. We have added fully automated diffusion tensor processing and tractography to our pipeline, routinely producing exquisite structural morphometric analyses and well as atlas-based tract extractions that are available for clinical patients on PACS. We have also recently implemented automated fiber quantification (AFQ) allowing extraction of a variety of diffusion metrics from regional white matter tracts for investigating diseases affecting brain white matter (**Figure 2**).

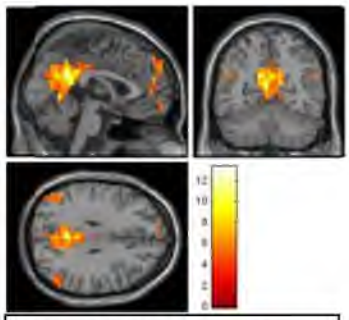


Figure 3. Group resting state seed-based DMN

G. Seed based network connectivity pipeline. We have implemented fully automated seed-based processing of resting state fMRI data for clinical and research studies. It operates from the point of data acquisition and uses labels defined in our wfu_pickatlas software or pre-defined user ROIs. **Figure 3** demonstrates a group default mode network (DMN) constructed from a study of 10 subjects using a posterior cingulate seed region of interest ($p < 0.05$ corrected).

H. Graph theoretical pipeline. We have also implemented a fully automated pipeline for voxel-wise as well as wfu_pickatlas label-based node definition graph theory

[redacted]

J. Automated NHP structural analysis pipeline. RIIPL investigators [redacted] and colleagues) have developed a fully automated structural image analysis pipeline for non-human primates (NHP). We have used this procedure successfully on a variety of NHP populations including vervets, cynomolgus and rhesus macaques. The pipeline generates exquisite NHP structural segmentations and mappings to the most detailed NHP atlas available. In comparison, the only other vervet atlases currently available have a maximum of 10 structures, and there are no available cynomolgus atlases. Additionally, currently available "automated" NHP skull-stripping and segmentation tools typically perform very poorly without manual interventions. Our implementation is fully automated, extremely robust across NHP species and brain positioning, and uses the most sophisticated morphometric analysis tools available, adapted for use with NHP (**Figures 6 and 7 and see Research Strategy, Figures 3 and 4**). The NHP atlases have been fully integrated into the wfu-pickatlas software to allow direct region-based hypothesis testing through SPM.

[redacted]

3. Translational Imaging Program Resources

The Translational Imaging Program at Wake Forest School of Medicine occupies space in several locations:

- [redacted] including an office suite for Bioinformatics, an image analysis training room, a video editing lab, plus cubicles for programmers and analysts
- [redacted]

There are several large bench laboratories and multiple small bench laboratories, which are used for the production of radionuclides and radiopharmaceuticals, an image analysis lab, and a cyclotron. These laboratories are well-equipped with current technologies. The ground floor and first floor of the [redacted] contain office suites for faculty and staff. Specific facilities are described below.

A. MRI Research Imaging

The 1,750 sq. ft. [redacted] facility is located [redacted] It is operated by dedicated MR registered technologists during normal business hours. The technologists have experience in all aspects of MR imaging research, including animal imaging. The facility is equipped with all necessary supplies, including resuscitation equipment.

The facility houses a Siemens MAGNETOM Skyra 3T MRI scanner with TIM technology operating on a D13 platform with the following features:

- 3T Siemens Skyra operating at D13 platform
- Gradient field strength of 45 mT/m, SR 200 T/m/s
- 70 cm open bore design, weight limit 500 lbs
- Capable of Advanced DTI, BOLD, Spectroscopy, ASL, Map-It (cartilage) and Advanced Cardiac Imaging
- Equipped with stimulation equipment for fMRI studies
- Various coils including 32 channel head coil, 20 channel head/neck, 18 channel body, 32 channel spine, 15 channel knee and surface loop coils.
- Contrast Power Injector

All image data are supplied to investigators in compliance with IRB and HIPAA regulations. Data can be downloaded to dedicated research image workstations for investigator analysis and/ or to be formatted for transmission to offsite reading centers.

B. Laboratories

Organic chemistry laboratory (860 sq. ft.): three fume hoods, two rotary evaporators, one Perkin-Elmer series 1600 FT-IR for characterization of synthesized compounds, a high-range vacuum pump, >5 HPLC systems attached with radioisotope and UV detectors for radiochemical synthesis, Varian GLC (TC and radiation detectors), and TLC scanner.

A second organic chemistry laboratory (478 sq. ft.): three chemical fume hoods, a rotary evaporator, two high vacuum pumps, and several routine laboratory instrumentation to perform chemical synthesis.

Metabolite analysis lab: Varian Analytical HPLC (attached with UV and radioisotope detectors) for metabolite analysis, three micro-centrifuges, a rotary evaporator, and a Packard Cobra II auto-gamma counter.

Radiochemistry laboratory (1,350 sq. ft.): Two Capintech Hot Cells, two Comecere hot cells, four mini-cells, and one GE [11C] methyl iodide synthesis box for radiochemistry. In an area remote from the hot cells and shielded fume is a laboratory containing three fume hoods, a shielded rotary evaporator, and a rotary chromatatron, and a laminar flow hood.

C. Imaging Informatics Resources

[redacted] oversees all phases of database design, development, and management. He is also responsible for administration of TeraRecon servers and workstations, and training investigators, faculty, and study coordinators in best methods of data collection and image analysis. He collaborates with investigators to determine image analysis requirements to achieve study/grant objectives. Equipment and software resources include the following.

TeraRecon Systems

TeraRecon AquariusNET servers

- Distributed 2-D/3-D/4-D real-time rendering and visualization on any windows PC via local network
- Total of 15TB of storage space for Medical Imaging in RAID5+1 configuration directly connected to servers
- Concurrently 3-D render ~36,000 images in real-time
- Render images from any modality in 3-D from a stack of 2-D DICOM images
- Virtual Endoscopy
- MPR, MIP, 3-D, 4-D
- Image fusion
- JPEG, AVI, and DICOM output

TeraRecon Aquarius workstations

- Advanced 2-D/3-D/4-D real-time rendering and visualization imaging workstation
- 500GB of direct attached storage space for storage of medical images
- Each workstation can concurrently render 3,400 images in real-time
- Render images from any modality in 3-D from a stack of 2-D DICOM images
- Volumetric, area, and distance measurement capabilities
- Advanced segmentation and analysis modules
- Virtual Endoscopy
- MPR, MIP, 3-D, 4-D
- Image fusion
- JPEG and AVI output

OsiriX

- 2-D/3-D/4-D advanced imaging software for Macintosh
- Volumetric, area, and distance measurement capabilities
- Advanced segmentation and analysis modules
- Open source software
- QuickTime Virtual Reality movies
    - Interactive movies for medical imaging
    - Embeddable movies into PowerPoint presentations
    - Embeddable movies into web pages

Mimics

- Advanced 2-D/3-D modeling software for PC
- Volumetric, area, and distance measurement capabilities
- Advanced segmentation and analysis modules
- Generates 3-D AutoCAD files for printing on 3-D printer

Amira

- Advanced 2-D/3-D modeling software for PC
- Volumetric, area, and distance measurement capabilities
- Advanced segmentation and analysis modules
- Generates 3-D autocad files for printing on 3-D printer

ImageJ

- Advanced imaging software
- Open source software
- Based on Java for various platforms of operating systems
- Advanced segmentation and analysis capabilities

LCModel

- Automatic quantification of in vivo proton MR spectra
- Fully developed over 15 years with spectra analyzed from a wide variety of scanners and field strengths at more than 400 sites

GE Advantage Workstations

- Advanced 2-D/3-D/4-D real-time rendering and visualization imaging workstation
- Render images from any modality in 3-D from a stack of 2-D DICOM images
- Volumetric, area, and distance measurement capabilities
- Advanced segmentation and analysis modules
- MPR, MIP, 3-D, 4-D

MIPAV

- Advanced imaging software
- Advanced segmentation and analysis capabilities

DICOM server

- Digital Imaging and Communications in Medicine
- Network storage of medical images from all modalities in DICOM format
- Imaging workstation and imaging modalities can send images in DICOM format for storage and retrieval from the DICOM server
- Image database for organization of medical images
- DCM4CHEE (www.dcm4che.org)
- PHP (www.php.net)

- MYSQL (www.mysql.com)
- Apache (www.apache.org)

Apple Workstations and Servers

- Blu-ray DVD backup
- Exabyte 320GB tape archive
- Audio/Video editing and encoding workstations
- Training videos and images generated on these workstations
- Website development
- Database development
- 3-D/4-D medical animation and illustration
- Final Cut video editing software and hardware
- ~30TB hard drive storage systems

Autodesk Maya 9

- Animation software for 3-D rendering
- Animate 3-D objects generated from CT and MR scans
- Rendering capabilities to 1080p

Medical Imaging Resource Center (MIRC) Software & Hardware

- Medical Imaging Resource Center
- WFBMC helped to develop
- Beta site
- Open source
- Software for secure image transmission using the Internet
- Coordinating and reading centers use MIRC to automate the sending and receiving of DICOM images from various hospitals and cities around the world
- Functionality to automatically de-identify medical images

RESOURCES: EQUIPMENT

For a description of the on-site equipment that will be used (and available based on need) during the course of the proposed studies, please the “Resources: Facilities and other Resources” section where it is described in conjunction with the associated facilities. No new equipment will be required to complete these studies.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Paul Middle Name Last Name*: Czoty Suffix:

Position/Title*: Associate Professor
Organization Name*: Wake Forest University Health Sciences
Department: Physiology_Pharmacology
Division:
Street1*: [redacted]
Street2:
City*: Winston-Salem
County: Forsyth
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27157-0001

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: pczoty@wakehealth.edu

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

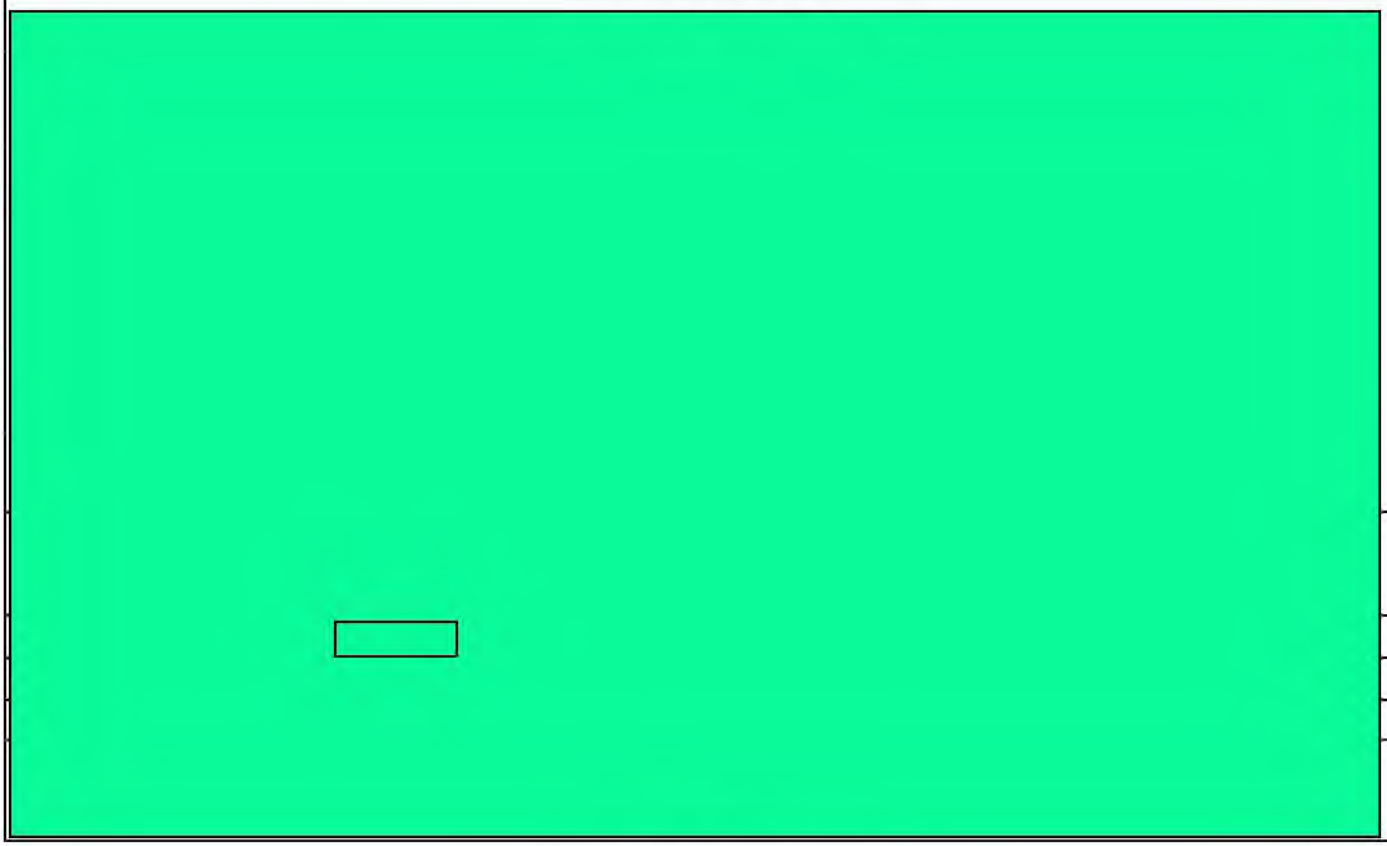
Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

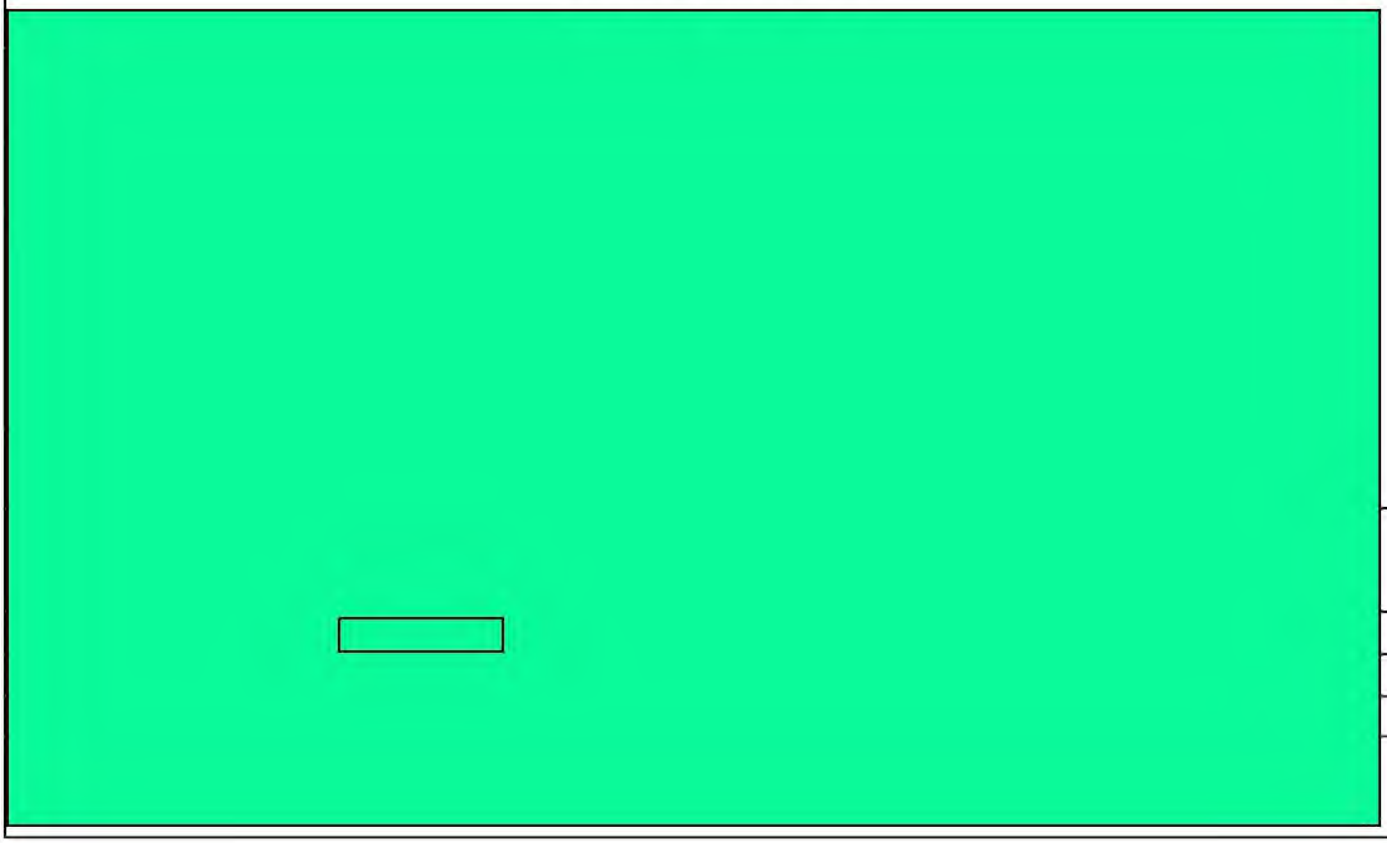
Attach Biographical Sketch*: File Name: Czoty_Bio.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person



PROFILE - Senior/Key Person



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

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2017 **End Date*:** 11-30-2018 **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.		Paul		Czoty		SubProject PI					17,250.00	2,588.00	19,838.00
2.						Co-Investigator					4,600.00	690.00	5,290.00
3.						Co-Investigator					9,255.00	1,388.00	10,643.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,771.00**

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						
1	Programmer	0.6			2,600.00	702.00	3,302.00
1	Research Assistant	5.88			30,776.00	8,310.00	39,086.00
1	Technician	9			28,500.00	8,835.00	37,335.00
1	Research Associate	1.2			5,500.00	1,485.00	6,985.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**86,708.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**122,479.00**

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

Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2017 **End Date*:** 11-30-2018 **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	

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**	

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

Tracking Number: GRANT12298582
Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2017 **End Date*:** 11-30-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	29,643.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	125,049.00
Total Other Direct Costs	**154,692.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**277,171.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Organized Research	55	277,171.00	152,444.00
		Total Indirect Costs	**152,444.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**429,615.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Proj2_BudgetJustification.pdf
(Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **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.		Paul		Czoty		SubProject PI					17,250.00	2,588.00	19,838.00
2.						Co-Investigator					4,600.00	690.00	5,290.00
3.						Co-Investigator					9,255.00	1,388.00	10,643.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,771.00**

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						
1	Programmer	0.6			2,600.00	702.00	3,302.00
1	Research Assistant	5.88			30,776.00	8,310.00	39,086.00
1	Technician	12			38,000.00	11,780.00	49,780.00
1	Research Associate	1.2			5,500.00	1,485.00	6,985.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**99,153.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**134,924.00**

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

Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **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	

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**	

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

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

ORGANIZATIONAL DUNS*: 9377279070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	29,323.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	86,828.00
Total Other Direct Costs	**116,151.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**251,075.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Organized Research	55	251,075.00	138,091.00
		Total Indirect Costs	**138,091.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**389,166.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Proj2_BudgetJustification.pdf
(Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **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.		Paul		Czoty		SubProject PI					17,250.00	2,588.00	19,838.00
2.						Co-Investigator					4,600.00	690.00	5,290.00
3.						Co-Investigator					9,255.00	1,388.00	10,643.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,771.00**

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						
1	Programmer	0.6			2,600.00	702.00	3,302.00
1	Research Assistant	5.88			30,776.00	8,310.00	39,086.00
1	Technician	12			38,000.00	11,780.00	49,780.00
1	Research Associate	1.2			5,500.00	1,485.00	6,985.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**99,153.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**134,924.00**

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

Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

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

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**	

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 9377279070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	8,300.00
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	
8. Other Costs	88,185.00
Total Other Direct Costs	**96,485.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**231,409.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Organized Research	55	231,409.00	127,275.00
		Total Indirect Costs	**127,275.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**358,684.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Proj2_BudgetJustification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12298582
Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **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.		Paul		Czoty		SubProject PI					17,250.00	2,588.00	19,838.00
2.						Co-Investigator					4,600.00	690.00	5,290.00
3.						Co-Investigator					9,255.00	1,388.00	10,643.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,771.00**

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						
1	Programmer	0.6			2,600.00	702.00	3,302.00
1	Research Assistant	5.88			30,776.00	8,310.00	39,086.00
1	Technician	12			38,000.00	11,780.00	49,780.00
1	Research Associate	1.2			5,500.00	1,485.00	6,985.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**99,153.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**134,924.00**

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

Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

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

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**	

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 9377279070000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	8,300.00
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	
8. Other Costs	89,587.00
Total Other Direct Costs	**97,887.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**232,811.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Organized Research	55	232,811.00	128,046.00
		Total Indirect Costs	**128,046.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**360,857.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Proj2_BudgetJustification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12298582
Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **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.		Paul		Czoty		SubProject PI					17,250.00	2,588.00	19,838.00
2.						Co-Investigator					4,600.00	690.00	5,290.00
3.						Co-Investigator					9,255.00	1,388.00	10,643.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,771.00**

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						
1	Programmer	0.6			2,600.00	702.00	3,302.00
1	Research Assistant	5.88			30,776.00	8,310.00	39,086.00
1	Technician	12			38,000.00	11,780.00	49,780.00
1	Research Associate	1.2			5,500.00	1,485.00	6,985.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**99,153.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**134,924.00**

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

Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

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

ORGANIZATIONAL DUNS*: 9377279070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Wake Forest University Health Sciences

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

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**	

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

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

ORGANIZATIONAL DUNS*: 9377279070000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Wake Forest University Health Sciences

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	12,652.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	91,032.00
Total Other Direct Costs	**103,684.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**238,608.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Organized Research	55	238,608.00	131,234.00
		Total Indirect Costs	**131,234.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**369,842.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Proj2_BudgetJustification.pdf
(Only attach one file.)

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

BUDGET JUSTIFICATION KEY PERSONNEL:

Paul Czoty, Ph.D., (effort = calendar months, Project Director) is an Associate Professor in the Department of Physiology and Pharmacology at Wake Forest School of Medicine (WFSM). He a behavioral neuropharmacologist with extensive expertise in all the techniques utilized in the proposed studies, having conducted nonhuman primate studies involving cocaine self-administration for more than 20 years and studies involving ethanol self-administration for the past 2 years. Dr. Czoty will oversee all phases of the experiments, including guiding the conduct of nonhuman primate behavioral and brain imaging studies, compliance with Institutional Animal Care and Use (IACUC) protocols, data analysis and preparation of the results for publication.

(effort = calendar months, Co-Investigator) is an Associate Professor in the Department of Physiology and Pharmacology. He is a primate neuroanatomist with over 20 years of experience in human and non-human primate neuroanatomy. has 12 years of multimodal imaging experience in NHPs and 6 years of experience with the ethanol self-administration paradigm. His group was the first to report how acute and chronic EtOH alters functional connectivity in NHPs (Telesford et al., 2013, 2015). will be a Co I on this project and will provide expertise and oversight for the MRI imaging component of the project, and assistance with the design and interpretation of the ethanol self-administration studies. will also assist in preparation of manuscripts to disseminate the results of this study.

(effort = calendar months, Co-Investigator) is an Associate Professor in the Department of Radiology at WFSM and Co-Leader of the Translational Imaging Program (TIP) of the WFSM Clinical and Translational Science Institute (CTSI). He will be responsible for overseeing all image processing and analysis for the proposed imaging studies.

effort= calendar months) is a Research Associate in the Department of Radiology and will assist with the MRI scans and maintain accurate records of the statistical data obtained from the scans.

(effort = calendar months) is an experienced research assistant with over 12 years of experience in the non-human primate self administration paradigm and image analysis techniques proposed in this project. She will be responsible for management of daily operations in the lab, oversight of the self administration paradigm and training and supervision of the technicians in self administration. She will manage budgets, acquire supplies, schedule scans and procedures and assist with panel training. She will also perform BEC and drinking behavior data analyses. She will assist with MRI acquisitions and data analysis, as well as manuscript preparation.

(effort= calendar months Year 1; calendar months years 2-5) Research Technician II. In conjunction with will be primarily responsible for the daily care, handling, and panel training of the monkeys. He has significant expertise with the ethanol self-administration paradigm and MRI procedures. The self administration paradigm requires that the animals are on the panels 22 hours per day, 7 days per week, which requires technical staffing seven days per week. This labor intensive paradigm requires appropriate technical staffing since the animals run 365 days per year.

(effort= calendar months) is a programmer in the Department of Radiology and maintain the database of MRI scans. He will prepare the necessary programs to review and generate measurements of the data obtained.

Subtotals Personnel: Year 1: $122,479; Year 2: $134,924; Year 3: $134,924; Year 4: $134,924; Year 5: $134,924.

Supplies:

New Self Administration Panel Components: The following items are required to construct and drive new 6 computer interfaced self administration panels. This is expense is split into the purchase of three panels in year 1 and 3 panels in year 2 for a total expense of $47,044 or $23,523 in years 1 and 2.

Category	Description	Price each	Total needed	Total Cost
Panels	metal panel	$ 310.00	6	$ 1,860.00
Panels	lights - 3 per spout, 2 light sets per panel	$ 59.00	36	$ 2,124.00
Panels	feeder - 1 per panel	$ 626.00	6	$ 3,756.00
Panels	bar pull - 1 per panel	$ 202.00	6	$ 1,212.00
Panels	poke response - 1 per panel	$ 120.00	6	$ 720.00
Panels	food cup - 1 per panel	$ 85.00	6	$ 510.00
Panels	spouts - 2 per panel	$ 20.00	12	$ 240.00
Panels	balance - 2 per panel	$ 900.00	12	$ 10,800.00
Panels	valves - 2 per panel	$ 65.00	12	$ 780.00
Panels	valve controller - 1 per 2 valves, 1 per panel	$ 600.00	6	$ 3,600.00
Control system	main computer, monitor and Lab View Software	$ 3,500.00	1	$ 3,500.00
Control system	data acquisition card - 1 per computer	$ 470.00	1	$ 470.00
Control system	switch module 1 per panel	$ 1,343.75	6	$ 8,062.50
Control system	input module with terminal - 1 per 32 inputs -- 32 inputs (2 per panel)	$ 1,062.00	1	$ 1,062.00
Control system	cabling - (2 balance + 1 panel cable) per panel	$ 150.00	18	$ 2,700.00
Control system	communication cards - 1 per 16 balances	$ 567.10	2	$ 1,134.20
Marble slabs	Balance slab, marble VWR 12568-006	$ 189.00	12	$ 2268.00
bottles	Carboy, Nalgene LDPE w/tubulation, 2L FS 02 962B	$ 67.21	12	$ 806.52
tubing	Nalgene, 180 Clear PVC tubing, 250' FS 14 176 90	$ 138.77	2	$ 277.54
disconnects	Quick-disconnect, hose barb body, 3/16"ID ColeParmer P-06 360-22	$ 5.25	48	$ 252.00
disconnects	Quick-disconnect, hose barb insert, 3/16"ID ColeParmer P-06 360-43	$ 5.25	48	$ 252.00
brass adapter	brass adapter for lixit (coupler)	$ 1.05	24	$ 25.20
brass adapter	brass adapter for tubing (hose barb)	$ 0.95	24	$ 22.80
carboys (lg)	20L Nalgene LDPE Carboy w/spigots FS 02 963C	$ 80.00	2	$ 160.00
Shelving	2 per cage rack	$ 75.00	6	$ 450.00
	Total Components for Construction and Operation of 6 Self Administration Panels:			**$ 47,044.76**

Refurbish 6 Existing Self Administration Panels: The existing panels are over 15 years old. In order to make the old and the proposed new panels software and electronics compatible the 6 existing panels must be upgraded. This is a one-time expense of $3,720.00 in year 1.

MRI scan supplies: This category includes i.v. fluids, syringes, gauze, iv sets, alcohol pads, anesthesia supplies (ketamine and anesthesia machine gases) and isolation materials to protect the MRI environment from contamination. Our experience indicates that these expenses average $75/monkey/scan.
Year 1: 12 scans x $75/scan = $900.
Year 2: 24 scans x $75/scan = $1800.
Year 3: 24 scans x $75/scan = $1800.
Year 4: 24 scans x $75/scan = $1800.
Year 5: 24 scans x $75/scan = $1800.

Ethanol is supplied via the self administration panel as a 4% solution in RO ddH20.
Year 1: $0.00; Year 2: $1,000; Year 3: $1,000; Year 4 $1,000; Year 5 $1,000.

PPE: This category covers the cost of gloves, gowns, masks and scrubs required for non human primate handling.
Year 1: $750; Year 2: $1,500; Year 3: $1,500; Year 4: $1,500; Year 5: $1,500.

Glassware/Plastics/Gas Chromatography Supplies: This category covers the necessary glassware, plastics and gases for the lab and for performing blood ethanol analyses.
Year 1: $750; Year 2: $1,500; Year 3: $1,500; Year 4: $1,500; Year 5: $1,500.

Replacement Panel Parts: The self administration panels are interacted with daily and some components require replacement after constant use. It has been our experience that it costs approximately $208.33 to refurbish a panel. This $2,500 expense is expected to be incurred annually in years 3 and 4.

Subtotal Supplies: Year 1: $29,643.00; Year 2: $29,323; Year 3: $8,300; Year 4: $8,300; Year 5: $5,800.

Other Expenses:
Research Services (Animal Purchase and transport; Scans, animal per diems and dietary)
Animal purchase fees: We have been quoted thru PIRS a purchase price of $5650 per animal for a total of $67,800. The associated crate charge is $75/animal for a total of $900. The associated shipping charge is $5,000. These expenses are incurred in year 1 of the grant.

MRI Scans: the cost structure for the 3T MRI is $1,450 per scan (scan, processing and analysis).
Year 1: 12 monkeys x 1 scan (baseline) = $17,400.
Year 2: 12 monkeys x 2 scans (post group housing, post induction) = $34,800.
Year 3: 12 monkeys x 2 scans (6M and 12M 22hr) = $34,800.
Year 4: 12 monkeys x 2 scans (post Abstinence, 4hr) = $34,800.
Year 5: 12 monkeys x 2 scans (new baseline, post intervention) monkeys = $34,800.

Animal Per Diems:
Year 1: 12 animals @ 10.14/day for 9 months = $33,949.
Year 2: 12 animals @ 10.44/day for 12 months = $45,728.
Year 3: 12 animals @ 10.75/day for 12 months = $47,085.
Year 4: 12 animals @ 11.07/day for 12 months = $48,487.
Year 5: 12 animals @ 11.40/day for 10 months = $49,932.

Banana Pellets: Animals are maintained on an isocaloric diet. The self administration panels dispense meals in the form of 1 gram nutritionally complete banana pellets manufactured by BioServ. These pellets are delivered via finger-poke activated pellet feeders on the self administration panels. Banana pellet expenses are $525/12 monkeys/month once the animals start panel training and continue for the duration of the self administration paradigm.
Year 1: 0
Year 2: 12 animals @ 12 months = $6,300.
Year 3: 12 animals @ 12 months = $6,300.
Year 4: 12 animals @ 12 months = $6,300.
Year 5: 12 animals @ 12 months = $6,300.

Subtotal Other Expenses: Year 1: $125,049; Year 2: $86,828; Year 3: $88,185; Year 4: $89,587; Year 5: $91,032.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		178,855.00
Section B, Other Personnel		483,320.00
Total Number Other Personnel	20	
Total Salary, Wages and Fringe Benefits (A+B)		662,175.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		568,899.00
1. Materials and Supplies	88,218.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	480,681.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		1,231,074.00
Section H, Indirect Costs		677,090.00
Section I, Total Direct and Indirect Costs (G + H)		1,908,164.00
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ● No

2. 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 proved scientific justification

3. *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)

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4. 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, please check the box indicating that one from the registry will be used:

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

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

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

*Previously Reported: ○ Yes ○ No

6. 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: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	Proj2_SpecificAims.pdf
3. Research Strategy*	Proj2_researchstrategy_final.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Proj2_VertAnimals_full.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	
14. Resource Sharing Plan(s)	Proj2_ResourceSharing_full.pdf
15. Authentication of Key Biological and/or Chemical Resources	Proj2_authentication_full.pdf
Appendix	
16. Appendix	

Tracking Number: GRANT12298582
Funding Opportunity Number: RFA-AA-17-001. Received Date: 2016-12-05T15:32:14.000-05:00

SPECIFIC AIMS

Alcohol use disorder persists as a costly public health problem that lacks widely effective medications and strategies for prevention. One obstacle to more effective treatments is the lack of understanding of factors that confer vulnerability to developing alcohol use disorders. Although it is known that social stress can drive alcohol use, the mechanisms underlying individual differences in vulnerability to social stress are not well understood. It is difficult to identify such factors in humans due to the inability to study subjects prior to alcohol exposure and other difficulties inherent in human subjects research. Laboratory animals, particularly nonhuman primate (NHP) models, are useful in this regard. In addition to being the closest animals to humans in terms of brain structure and function, monkeys have an extensive social repertoire that makes them ideal animal subjects for studying the interaction between social variables and sensitivity to alcohol. In NHP social groups, monkeys that occupy lower ranks (i.e. subordinate monkeys) have been found to be more sensitive than dominant-ranked monkeys to the reinforcing effects of abused drugs, including ethanol (EtOH; Morgan et al. 2002; Czoty et al. 2004; McKenzie-Quirk and Miczek 2008). Experiments in this project will compliment and extend findings in rodents and humans regarding the brain mechanisms underlying vulnerability to developing alcohol use disorders. These studies in socially housed cynomolgus monkeys will determine the effects of chronic social stress and enrichment on EtOH drinking and on the ability of putative medications to decrease EtOH consumption. Parallel brain imaging studies will characterize the effects of social rank on brain structure and function and will determine how these variables change during chronic EtOH consumption in the absence and presence of putative pharmacotherapies. These data will provide novel insight into the neurobiological substrates that underlie vulnerability to EtOH reinforcement, identifying potential targets for novel medications. The Specific Aims are:

1. **To characterize the effects of social stress on long-term EtOH consumption.** Studies under this Aim will characterize the effects of social rank on initial and long-term EtOH consumption. 12 monkeys will be group-housed (4 per pen) and social ranks will be determined. Next, monkeys will be induced to drink EtOH using well-established procedures. Subsequently, they will have free access to EtOH [redacted] (Grant et al. 2008). We hypothesize that EtOH consumption will be higher initially, and increase over time, in subordinate compared to dominant monkeys. Subsequently, EtOH access will be discontinued [redacted] Monthly tests will assess monkeys' reactivity to cues predictive of EtOH availability using an animal model of relapse. We expect subordinate monkeys to be more reactive to these cues.

2. **To determine the effects of pharmacological interventions on EtOH consumption.** Studies under this aim will examine whether amounts and patterns of EtOH drinking are differentially sensitive to pharmacological manipulations based on social rank. We will examine drinking chronic treatment with putative medications for alcohol use disorder. These drugs will be determined in conjunction with rodent studies (Projects 3 & 4) that will identify specific substrates that show promise as mediators of enhanced vulnerability to the abuse-related effects of EtOH. We hypothesize that subordinate monkeys will be less sensitive to the effects of treatments designed to decrease EtOH consumption.

3. **To identify changes in brain structure and function that mediates stress-induced vulnerability to EtOH reinforcement.** In parallel with Aims 1 and 2, brain imaging will be used to identify social rank-related differences in brain structure and function that precede and are produced by environmental and pharmacological manipulations. Structural MRI will be used to characterize social rank-related differences in cortical structure and resting state fMRI will be used to generate whole-brain functional networks. Scans will be scheduled to address specific experimental questions at several time points during these studies. These include: before and after social hierarchy formation; after induction of EtOH drinking; [redacted] from EtOH and after chronic treatment with putative pharmacotherapies. Targeted brain areas will be selected in consultation with the findings in human subjects which will use an overlapping set of imaging modalities (Project 1). We hypothesize that subordinate monkeys, who will be more vulnerable to the initial effects of EtOH and who will consume more EtOH over time, will show the greatest changes in brain structure and function.

Research Strategy

(A) Significance

A.1. Scientific Premise. The overarching scientific premise of the WF-TARC is that the neurobiological substrates that contribute to vulnerability and resilience to alcohol use disorders (AUD) are not fully understood. Studies using non-human primate (NHP) subjects have advantages that make them a critical part of a comprehensive, translational approach to addressing this topic. NHP studies can overcome limitations of experiments in both human subjects (uncertainty drinking history, polysubstance abuse and comorbid psychiatric disorders; necessity of cross-sectional designs) and rodents (short life span, differences in neuroarchitecture). Limitations of NHP studies are mitigated by the close relationship of this Project with others in the WF-TARC. Results of these studies, which use a well-described, highly translational NHP model of social stress and vulnerability to substance abuse, will improve scientific knowledge and advance the field by determining the temporal and anatomical profile of brain changes that underlie vulnerability to developing AUD. This knowledge will help identify novel targets for more effective interventions targeted to the most vulnerable individuals.

A.2. Significance of research question and role of non-human primate studies. The recent Surgeon General's Report on addiction [1] noted that AUD contributes to 88,000 deaths and costs the US ~$249 billion each year [2,3]. Considering the complex nature of AUD, it is not surprising that existing medications are not universally effective [4,5]. There exists a critical need to better understand factors that confer vulnerability to AUD and for novel medications targeting these subpopulations; animal models can provide valuable information. Although knowledge can be gained about the neuropharmacology of ethanol (EtOH) in rodents, the ability to generate clinically relevant phenotypes related to long-term drinking is limited in these species. NHPs are the animal model most similar to humans in terms of genetics, neuroanatomy and pharmacokinetics [6,7]. NHP models are highly translational with respect to the neurobiological consequences of long-term alcohol use. In the present context, a critical advantage is that longitudinal studies can be conducted in the same subjects starting when they are treatment-naïve. The development of AUD is not an acute process. Rather, in vulnerable individuals, initial experimentation sets into motion a cascade of brain changes, eventually leading to neuroadaptative processes that facilitate addiction [8,9]. Moreover, individuals presenting for treatment have accumulated years of alcohol exposure. Thus, the study of how *long-term* alcohol use and vulnerability factors interact is critical. EtOH produces widespread changes in brain structure and function, the timing and relative importance of which are not fully understood. The importance of identifying mechanisms of alcohol's action and pathology was emphasized in NIAAA's recent strategic plan. The translational studies described in this application combine EtOH self-administration and state-of-the-art brain imaging methods to determine, in a NHP model of AUD vulnerability, the neurobiological factors that confer vulnerability to AUD, changes in brain structure and function during long-term EtOH self-administration and abstinence as well as differences between vulnerable and resilient individuals in sensitivity to putative medications for AUD.

A.3. NHP social housing: a model of human disease vulnerability. For decades, NHP social groups have proven useful as models of human susceptibility and resistance to disease. Biological differences among NHPs in different positions of the social hierarchy have been linked to predictable variation in physiology, neurobiology and behavior. For example, socially subordinate monkeys are more susceptible than dominant monkeys to immune, cardiovascular and reproductive dysfunction [10-14]. The influence of social rank on health in NHPs parallels the inverse relationship between human socioeconomic status and susceptibility to disease [15]. One explanation of the physiological and neurobiological differences between high (dominant) and low-ranking (subordinate) monkeys involves a greater amount of stress experienced by subordinates, as encompassed by the concept of allostatic load [16]. Understanding how the neurobiological mechanisms of these effects influence sensitivity to AUD would enhance the translational relevance of preclinical studies [17]. For example, we have conceptualized the dominance hierarchy as a continuum from environmental enrichment in dominant monkeys to chronic social stress in subordinates [18,19]. Importantly, social rank-related differences extend to models of substance abuse. For example, Project Director (PD) Dr. Paul Czoty has been a Co-I on studies of the influence of social rank on sensitivity to cocaine. Becoming dominant leads to increased dopamine D2-like receptor availability as measured with PET imaging and lower cocaine self-administration compared to subordinates [20-24]. These relationships parallel human data [25, 26]. Studies in animals have also shown that social stress increases EtOH consumption [27-29]. For example, NHP studies have shown inverse relationships between dominance rank and alcohol intake [30-33]. The proposed studies

will compare behavior, brain structure and function as well as sensitivity to putative pharmacotherapies in dominant and subordinate subjects to better understand the biological basis of AUD vulnerability.

A.4. NHP models of AUD. For 50 years, NHP models of human alcohol drinking have been a critical part of efforts to understand the biological basis of AUD. Early studies used intravenous EtOH self-administration [34,35], but investigators soon developed procedures to achieve stable oral EtOH consumption [see 36]. Supporting the translational value of these models, increases in drinking are observed after exposure to stress [28,37], including social subordination [30-33]. The proposed studies utilize the self-administration model developed by Grant et al. [38]. Under this procedure, monkeys are trained to drink EtOH using schedule induction. Subsequently, they are provided access to 4% EtOH 22 hours/day. The translational relevance of this model has been clearly established; similarities with human alcohol drinking have been demonstrated with regard to biological characteristics [38], the observance of drinking phenotypes (low, binge, heavy, very heavy [39]) and effects on stress-related hormones and circulating proteins [33,40]. The proposed studies will implement this model to longitudinally compare dominant (resilient) and subordinate (vulnerable) monkeys' trajectories of EtOH self-administration over one year using between-group and within-subject analyses (**Aim 1**). In addition, we will assess differences in EtOH seeking during abstinence. These data will provide critical clinically relevant information regarding whether characteristics that confer vulnerability also influence recovery once EtOH use is discontinued. Parallel brain imaging studies will characterize the mechanisms underlying these phenotypes by identifying differences in brain structure and function that exist before, develop during and persist after long-term EtOH drinking.

Identifying mechanisms by which social stress confers vulnerability to AUD is an important step in improving treatments. A second step is to develop interventions to target vulnerable phenotypes. Thus, **Aim 2** is to compare the sensitivity of dominant and subordinate monkeys to the effects of chronic treatment with putative AUD medications. Specific drugs will be selected based on preliminary data and the results of WF-TARC rodent Projects (3 and 4), which will identify promising targets and suggest specific compounds with potential for reducing EtOH drinking. Our NHP studies will expand on rodent data by implementing a highly translational model of pharmacotherapy evaluation [41] that incorporates aspects of human substance abuse treatment (C.2b.2). Clinically relevant features of the model, developed in part by the PD, include: (1) subjects with an extensive drug use history, (2) an individual-subject design wherein the dose of treatment drug is adjusted based on effect as opposed to a group design in which all monkeys receive identical treatment regimens regardless of effect and (3) concurrent monitoring of food-maintained responding to exclude the possibility that decreases in EtOH drinking are due to non-selective behavioral disruptions. Results of studies conducted by the PD and collaborators demonstrate good predictive validity of this approach with respect to the results of clinical trials [41-43].

A.5. Alterations in brain structure and function during long-term EtOH use. Brain imaging studies have documented differences between alcoholics and controls in several measures of brain structure and function. Structural magnetic resonance imaging (MRI) data show that alcoholics have lower volumes of cortical gray matter (GM) and white matter (WM) than controls [44-46] in agreement with post-mortem analyses [47,48]. Frontal areas such as the medial prefrontal cortex (mPFC) appear to be most vulnerable [49-51]. WM and GM loss has been linked to cognitive and memory deficits [52]. Diffusion imaging studies have provided additional understanding of these changes, providing evidence for neuronal death and other structural abnormalities, particularly in the frontal cortex and tracts that connect it to other brain areas [53-60]. As with structural MRI, diffusion imaging has linked these deficits to dysfunction in cognitive performance and memory [61-65].

As an important complement to structural imaging, functional MRI (fMRI) techniques have been developed to study *connectivity*: temporally correlated activity in distinct brain areas that define integrated networks [66-67]. Alcohol research often utilizes a region-of-interest (ROI) analysis to evaluate the impact of EtOH on structure and function that may miss broader effects resulting from the vast interconnectivity in brain networks. One such network, the default mode network (DMN [68]), is a set of brain regions that displays correlated activity when the brain is not involved in goal-directed activity [69]. In humans, alcohol use disrupts functional connectivity in the DMN and other brain networks [70-73]. For example, [redacted] and colleagues recently showed that moderate-to-heavy drinkers exhibited decreased connectivity in the central executive network during a cognitive task [74]. In the past decade, studies of functional connectivity have been extended to NHP, which show a high degree of similarity to humans in identified brain networks including the DMN [75], and to NHP addiction models [75-77]. For example, our laboratory in collaboration

with [redacted] has characterized brain networks, as measured with blood oxygen-dependent (BOLD) fMRI, in vervet monkeys who underwent the EtOH self-administration procedure to be used in the proposed studies [76]. After 15 months of daily EtOH consumption, monkeys characterized as heavy drinkers showed differences in network organization compared to light and non-drinkers. We will use structural MRI and fMRI techniques throughout the course of the proposed studies (**Aim 3**). These experiments will provide unique, highly translatable data regarding: (1) effects of social stress on brain structure and function, (2) brain differences between EtOH-vulnerable and -resistant phenotypes, (3) effects of long-term oral EtOH self-administration and (4) subsequent abstinence on these processes and (5) effects of chronic administration of treatments that effectively reduce EtOH self-administration on these neurobiological variables.

(B) Innovation Innovative aspects of the proposed studies include: **(1)** the use of NHP, which have similar neuroanatomy compared to humans; **(2)** the use of naturalistic social variables to generate an EtOH-vulnerable phenotype; **(3)** longitudinal studies, making possible within-subject evaluation of manipulations starting before the subject is exposed to EtOH; **(4)** the use of multi-modal MRI to obtain structural and functional imaging data from the same NHP subjects **(5)** the combination of these imaging studies with long-term oral EtOH self-administration and **(6)** the application of whole-brain network analyses to these NHP data.

(C) Approach

C.1. Preliminary Data

[redacted]

C.2. Experimental Design

C.2a. Specific Experiments. Fig. 9 depicts the 5-year timeline of this project. Briefly, once social hierarchies stabilize, monkeys will be trained to use the operant panel and will be induced to drink EtOH. Next, the dominant and 6 subordinate monkeys will drink EtOH 22-hr/day, 4 days/week for one year. At that point EtOH access will be discontinued for 6 months during which EtOH seeking will be assessed as a model of vulnerability to relapse (**Aim 1).** Next, EtOH access will be decreased to 6 hrs/day and we will evaluate potential pharmacotherapies (suggested by Projects 3 and 4) administered chronically (**Aim 2**). MRI scans will occur at predetermined points in the timeline to address specific research questions (**Aim 3**).

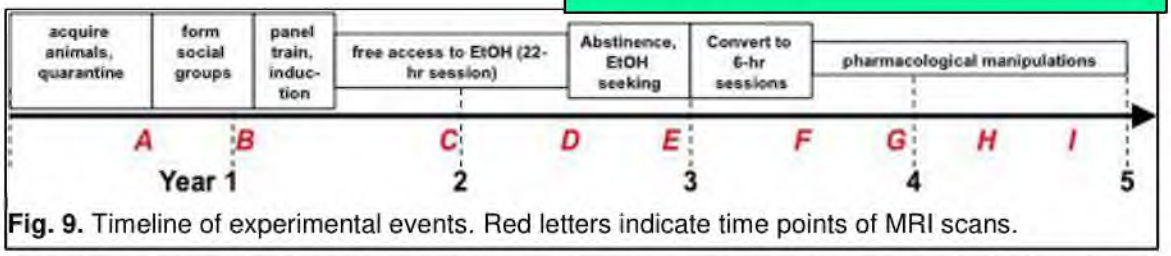


Fig. 9. Timeline of experimental events. Red letters indicate time points of MRI scans.

C.2a.1. Specific Aim 1. EtOH drinking in socially dominant and subordinate monkeys. The overarching goal of the WF-TARC is to characterize the neurobiological basis of vulnerability to AUD. To generate a group of subjects that model a vulnerable population, this Project will utilize socially housed cynomolgus monkeys. When housed in groups of 4, a dominance hierarchy develops that is linear and transitive [85,86]. Lower-ranked (subordinate) monkeys have been shown to be more sensitive to the behavioral effects of stimulants [20,22,87] and to consume greater amounts of alcohol [30-33]. This approach is analogous to Project 4 which uses social isolation to generate rodents with an EtOH-vulnerable phenotype.

Experiment 1.1. Induction of EtOH drinking. Studies showing rank-related differences in EtOH drinking have been conducted in monkeys with extensive EtOH-drinking histories. Whether dominant and subordinate monkeys differ during initial EtOH exposure is unknown. During induction, the speed at which a monkey reaches the daily maximum EtOH intake (i.e. a "sipping" vs. "gulping" pattern) predicts light vs. heavy drinking during subsequent free-access conditions [78]. will be constructed and for three months weekly observations will be conducted to determine each animal's social rank (C.2b.1). Once hierarchies have stabilized, monkeys will be trained to use the operant panel, then will undergo the 15-week induction procedure (C.2b.2). Following completion of this regimen, water will be substituted for EtOH (extinction) to ensure that EtOH functions as a reinforcer. On days that EtOH sessions occur, monkeys will earn all daily food during the session; on other days, they will be separated into quadrants of the cage for two hours to eat. Monkeys will be socially housed during this experiment. We hypothesize that subordinate monkeys will be more likely to be characterized as "gulpers"—the phenotype associated with later heavy drinking—compared to dominant monkeys, establishing their vulnerable phenotype.

Experiment 1.2. Long-term EtOH consumption. Once induction is complete, monkeys will be provided free access to water and EtOH , beginning at 11:00 a.m. On the remaining monkeys will be socially housed except to eat. These parameters allow for extensive EtOH self-administration with sufficient social interaction to maintain the hierarchy within each social group. Monkeys will also self-administer their daily allotment of food in the form of three daily "meals." At each meal, monkeys will self-administer one third of their daily pellet allotment. Meals will occur at the start of the drinking session and after 2 and 4 hours have elapsed. The primary dependent variable will be EtOH intake (g/kg) each week. We hypothesize that subordinates will drink more EtOH initially and will increase their drinking over time compared to dominant monkeys who will maintain constant, lower average daily intakes over the year.

Experiment 1.3. EtOH seeking during abstinence. After 12 months, drinking sessions will be discontinued and monkeys will be socially housed except to eat. Every 3 weeks for 6 months, monkeys will be separated and

tested in a 1-hr drinking session during which water will be available from both spouts (extinction conditions). EtOH-associated stimuli will be illuminated and the amount of water consumed from the EtOH-associated spout will be used as a measure of EtOH seeking to model craving in abstinent humans. We expect subordinates to consume more water initially and, although both groups will decrease consumption over time, we expect this extinction to be slower and/or less complete in subordinate monkeys.

Data analysis. Expt. 1.1: During induction, we will record several variables shown to predict subsequent heavy drinking under this model (see [78]) including number of "drinks" (continuous consumption with <5 sec between events) and "bouts" (consumption of 1.5 g/kg without a 5-min lapse). Paired *t*-tests will be used to compare dominant and subordinate monkeys across these measures. Expt. 1.2 & 1.3: The primary dependent variables will be weekly water and EtOH intake (Expt 1.2) or the water intake from the spout from which monkeys previously drank EtOH (Expt 1.3.). Mean data for each group (dominant vs. subordinate) will be analyzed using 2-way repeated-measures analysis of variance (RM ANOVA) with group and time (week) as factors, with post-hoc multiple comparisons testing to determine the weeks at which significant group differences are observed. Because EtOH intake is monitored continuously with sub-second resolution, we will be able to perform extensive secondary analyses of drinking rates and patterns (e.g. [38,78]).

Potential pitfalls and contingencies. We have a great deal of experience studying social housing in NHP and with this EtOH drinking model and do not anticipate any obstacles. If we do not see differences between dominant and subordinate monkeys during induction, we expect Expt. 1.2 to reveal that a longer history of EtOH drinking is required for group differences to emerge. In Expt. 1.3, it is possible that EtOH seeking may differ in monkeys that have similar lifetime EtOH intakes. If so, this would represent an interesting point of crossover with Project 1 which expects to see differences in alcohol craving despite similar EtOH histories. We would then inspect our (and Project 1's) imaging data to determine whether structural or functional characteristics differ according to EtOH seeking (/craving) as opposed to lifetime or mean daily EtOH intake.

C.2a.2. Specific Aim 2. Effects of potential pharmacotherapies on EtOH drinking. Although many neurotransmitter systems have been investigated in developing novel medications, only one drug has received FDA approval in the last 32 years. In Years 4 and 5 of this grant, the focus will shift from differentiating chronic EtOH drinking to measuring of the efficacy of putative medications in vulnerable and resistant populations. For these experiments, EtOH access will be decreased from 22 to 6 hrs/day (5 days/week). Shortening the session limits the possibility that an immediate drug-induced decrease in drinking will be obscured by compensatory increases after the drug effect has worn off. In addition, the shorter session reduces inter-subject variability in daily EtOH intake (Fig. 1.), which is more amenable to the assessment of treatment effects. The procedure for chronic drug testing is described in C.2b.2. Identification of a drug dose that decreases daily EtOH intake selectively (without affecting food-maintained responding) for 4 weeks will represent a positive result. At that point, treatment will continue and a MRI scan will be conducted (Expt 3.4). After the scan, treatment will be discontinued and monkeys will drink EtOH for ~3 weeks before another drug is tested. We anticipate testing 3 drugs under this Aim. Our strategy is to begin by testing [redacted] based on our preliminary data (Fig. 3) and then test the most promising drugs that emerge from the rodent studies (Projects 3 and 4).

Experiment 2.1. [redacted] Brain receptors for the [redacted] are among the targets suggested as having therapeutic potential for AUD [88,89]. Research in the past decade has characterized their potential as targets for medications to treat a number of psychiatric conditions [90].

[redacted]

Experiment 2.2. Potential medications suggested by WF-TARC rodent projects. Projects 3 and 4 of the Center will characterize the precise neurobiological differences underlying phenotypic vulnerability to EtOH in rodent models. Project 3 will characterize molecular mechanisms that underlie vulnerability in adolescents with a focus on glutamatergic and GABAergic adaptations in the basolateral amygdala (BLA). Project 4 will use well-established electrophysiological and neurochemical methods to examine adaptations in the BLA and other areas that occur during social isolation, a manipulation that results in increased EtOH self-administration. Project 4 will also directly manipulate relevant brain circuits using DREADD technology. These projects will identify specific substrates that can be targeted by potential AUD medications; likely targets include kappa opioid, glutamate and GABA receptors and elements of the mammalian target of rapamycin (mTOR) signaling pathway. Several such compounds will be evaluated in rodent models. In Expt. 2.2 we will extend assessment of the most promising of these compounds to NHP. Drugs will be administered chronically as described in C.2b.2. It is also possible that Project 1, which will determine mechanisms underlying the ability of mindfulness meditation to reduce alcohol craving, may identify brain circuits that can be targeted pharmacologically.

Data analysis. Each dose of a test compound will be administered daily for [redacted] The primary dependent variable will be mean EtOH intake (g/kg) over the last three days of each of treatment week. Data will be analyzed using a 3-way RM ANOVA with group (dominant vs. subordinate), week and drug dose as factors.

Potential pitfalls and contingencies. It is possible that, as occurs clinically, we will see individual differences in sensitivity to treatment drugs; a drug may produce qualitatively similar trajectories in all subjects, but at different doses. If this occurs we will consult with biostatistician Dr. Sean Simpson (WF-TARC Administrative Core) to perform more sophisticated analyses using multilevel mixed models [106]. Between the opioids and drugs mentioned above, we expect to have ample compounds to test even if some generate negative data.

C.2a.3. Specific Aim 3. Alterations in brain structure and function mediating vulnerability to EtOH reinforcement. A critical premise of the WF-TARC is that a thorough understanding of the specific brain changes that render individuals vulnerable to AUD will have far-reaching clinical implications. Studies under Aim 3 will use state-of-the-art imaging techniques to track changes in brain structure and function across all stages of the project (Fig. 9). NHP studies have the advantage of enabling a precise characterization of the temporal and topographic nature of brain changes starting from the EtOH-naïve state, which is not possible in human subjects that must rely on cross-sectional designs. This will allow us to assess whether baseline differences exist between groups that model AUD-vulnerable and -resilient populations (subordinate and dominant monkeys, respectively). In addition, we will characterize changes in brain structure and function over one year of EtOH consumption and 6 months of abstinence. We will also determine the functional effects of chronic treatment with drugs that selectively decrease EtOH drinking. Imaging modalities were selected in close consultation with Project 1 to maximize translation. Our comprehensive approach includes an analysis of T1-weighted structural images, diffusion imaging, CBF and resting-state fMRI. This strategy positions us to longitudinally track how brain changes contribute to vulnerability or resilience to the effects of EtOH. Although global measures will be collected during each scanning session, we will focus on brain regions and networks that parallel those in other WF-TARC projects (particularly Project 1), with extensive secondary analyses to follow. Areas of primary focus for each modality include GM nodes specifically identified as critical in EtOH vulnerability and withdrawal in preliminary studies in Projects 1, 3 and 4 of the WF-TARC:

- *Structural morphomerty-based MRI*: GM volumes in mPFC, hippocampus, amygdala, ventral striatum
- *Diffusion kurtosis imaging*: GM microstructure in the above-listed areas
- *CBF* & resting-state BOLD *fMRI*: blood flow within the above-listed areas and network connectivity within the mPFC and from the mPFC to regions in the DMN, ventral striatum and amygdala.

Experiment 3.1. Effects of social housing on brain structure and function. The utility of the NHP social hierarchy as a model of vulnerability and resilience to disease has led to much investigation of the biological basis of rank-related differences. For example, we characterized differences in gonadal and stress hormones across social ranks [86;107-109]. Despite their enormous potential, advanced brain imaging techniques have been used only rarely [20; 22, 23;110-114]. In this experiment, we will compare data from a scan that occurs while monkeys are individually housed **(A)** one that occurs ~3 months after group establishment, once social hierarchies have stabilized **(B)**. We hypothesize that individual differences in GM volume and functional

connectivity will exist at baseline that predict whether animals eventually become dominant or subordinate. We hypothesize that GM volume and connectivity will correlate with social status [112] in some of the same brain regions (amygdala, striatum) that show altered connectivity during abstinence in moderate-heavy drinking humans (Project 1). This hypothesis is supported by previous imaging studies that demonstrated a positive relationship between amygdala volume and social networks in humans [115] and NHP [110].

Experiment 3.2. Structural and functional changes during long-term EtOH self-administration. It is clear that chronic EtOH use alters brain structure and function [61,116-117]. For example, postmortem and neuroimaging studies have reported loss of cortical GM and WM volume [44-49]. However, the time course of these changes remains unclear in part because much of the evidence is derived from studies in individuals who have abused alcohol for decades. NHP models provide the ability to determine the time course of changes since measures can be captured in the naïve state and tracked over years. In previous collaborations with other WF-TARC investigators, monkeys who drank EtOH under the proposed drinking model for 15 months showed many genomic, molecular and functional changes [e.g. 118-121] including alterations in brain network organization identified by resting-state BOLD fMRI [76]. Cortical GM and WM loss occurs within 6 months [122]. Using MEG, we observed altered power in multiple cortical and subcortical nodes including the amygdala after 15 months of drinking (Rowland et al. under review). More recent MEG studies in the 6 monkeys currently in the laboratory suggest that brain function is altered after only 3 months of exposure in a manner dependent on rate of EtOH intake (Fig. 2). These findings highlight the importance of tracking EtOH-induced adaptations in brain activity from the naïve state through early and chronic use. Expt 3.2 will track changes in brain structure and function over one year of EtOH access in vulnerable and resilient monkeys. We hypothesize that early in the drinking history, alterations in CBF (ASL) and functional connectivity (resting-state BOLD) will precede early microstructural diffusion (DKI) and later macrostructural volumetric (T1-weighted) changes, and that frontal brain regions such as the mPFC will be most vulnerable. These hypotheses are supported by our recent MEG data showing altered resting-state activity after induction (Fig. 2) and decreased GM volume in frontal regions [122] after 6 months of daily drinking under the proposed drinking model.

Following the second series of scans under Expt. 3.1 (**B**), all animals will be trained on operant panels and will undergo EtOH induction (C.2b.2). Next, monkeys will be allowed EtOH access via the panel for 22 hr/day, 4 days/week. Monkeys will be re-scanned after 6 (**C**) and 12 (**D**) months to assess the impact of chronic EtOH exposure on brain structure and function. Preliminary Project 1 cross-sectional data show high connectivity within PFC regions and low connectivity to the posterior DMN during normal drinking. An advantage of our NHP model is that we can characterize these networks before animals are exposed to EtOH and relate changes in brain structure and connectivity to EtOH intake, blood EtOH concentration (BEC) and other dependent variables. We hypothesize that heavier drinkers (i.e. subordinate monkeys) will exhibit network changes in structure similar to the subjects in Project 1. We hypothesize that resting-state activity will be affected in an intake-dependent fashion, such that heavier drinkers will exhibit more profound disruptions in functional connectivity compared to lighter drinkers. This intake-dependent effect on network structure is supported by our previous fMRI study in monkeys [76].

Experiment 3.3. Recovery of EtOH-induced brain changes during abstinence. During abstinence from EtOH, WM and GM loss, and associated motor and cognitive function, begin to recover over weeks to months [115, 123-129]. However, studies of WM microstructure indicate that some deficits persist beyond 6-12 months of abstinence [130,131]. Although studies in human alcoholics are informative, achieving a clearer characterization of the magnitude and time course of recovery is hindered by uncertainty and variability in durations of abstinence within and across studies and other limitations inherent in human studies. Expt. 3.3 will determine the effects of 6 months of abstinence from EtOH in monkeys with a >1 year history of EtOH consumption by comparing a scan acquired at that time point (**E**) to each monkeys' 12-month scan (**D**) and EtOH-naïve scan (**B**). These data will provide insight into whether brain processes that underlie vulnerability also affect brain recovery during abstinence. Preliminary data from Project 1 showed trends for differences in connectivity between mPFC, ventral striatum and amygdala in high vs. low alcohol cravers. We hypothesize that decreases in GM volume and microstructural integrity will be greatest in the heaviest drinking monkeys but that this phenotype will also exhibit the greatest recovery during abstinence.

Experiment 3.4. Effects of putative AUD pharmacotherapies on functional connectivity. Aim 2 will identify drugs

that selectively decrease EtOH drinking. Expt. 3.4 consists of parallel imaging experiments. Beyond the basic-science benefit of identifying brain regions and processes that mediate EtOH reinforcement, characterizing the precise effects of a successful medication on brain function will have several benefits. First, it will reveal pre-existing neurofunctional characteristics that may serve as biomarkers to direct the choice of interventions in specific populations. Second, it may suggest specific brain processes that underlie treatment success. These data will be particularly compelling when compared with those of Project 1, which will characterize the effects of a non-pharmacological treatment, mindfulness meditation, on alcohol craving. Thus, these data will also aid the design of future medications. It is unlikely that short-term drug treatment will impact structural measures but we expect that CBF in key regions and functional connectivity in specific circuitry will be affected. Thus, we will focus our analyses on functional measures, but still collect structural data for future analyses. Once monkeys are drinking stable daily intakes under the 6-hr regimen of EtOH access, a scan (**F**) will be performed to serve as a new baseline for subsequent scans. As described in C.2a.2., a positive result is defined as a decrease in EtOH intake that is sustained for 28 days. At this point, a follow up scan will occur. We anticipate being able to scan each monkey after two different successful treatments (**G**, **H**, **I** in Fig. 4), although one of these scans may need to serve as a new baseline if the first treatment produces profound changes. We hypothesize that successful pharmacotherapies that effectively reduce EtOH self-administration will produce discrete patterns of effects on important regions like the mPFC and functional networks; effects may differ depending on the pharmacological mechanism of action of the treatment drug. We hypothesize that reduction in intake will remediate EtOH-induced network changes in a similar fashion to what is observed during abstinence. Scan **F** will be compared to the abstinence scan (**E**) to assess whether recovery of EtOH-induced brain changes during abstinence are reversed by resumption of drinking.

Data analysis. Image analysis methods are described in C.2c.4. Statistical analyses of these data will be conducted under the guidance of biostatistician Dr. Simpson (Administrative Core, Project 1 Co-I). Primary dependent variables will be GM volumes, mean kurtosis, CBF in ROIs selected *a priori* (see above), as well as intra- and extra-network connectivity of the DMN including to the mPFC. Imaging data obtained across all 5 years of the study (Fig. 9) will total 9 scans on 12 subjects. Changes in structural/functional MRI dependent measures will be examined using linear mixed effects models for longitudinal data [132], with non-normal data transformed if needed. Models will be fit separately for each MRI measure and brain region. Fixed effects will include group (dominant vs. subordinate) and time. Main effects and interactions will be tested using F-tests [133]. Least square mean estimates and standard errors of differences in MRI dependent measures between groups at different time periods will be presented. We anticipate between-group effect sizes to be smallest for measures of GM volume because they may require longer exposure to develop compared to microstructural (e.g., mean kurtosis) and functional (CBF and connectivity) measures. Thus we have computed statistical power for GM volumetric data. We anticipate having 80% power to detect main effect differences between groups for the targeted brain ROIs across a range of repeated-measures correlations given 9 scans for 12 subjects [see **Vertebrate Animals** for detailed calculations].

Additional analyses relating behavioral and imaging measures: Multivariate growth curve models [134] will be used to examine how levels of behavioral variables (e.g. EtOH consumption) are related to levels of structural and fMRI dependent measures (e.g. are differences in DMN connectivity related to EtOH consumption), as well as how the magnitude of change in behavioral measures are related to the magnitude of change in brain structure/function across subjects (e.g. are rates of change in drinking and DMN connectivity related). These models will also allow us to examine whether behavioral brain measures vary together over time within-subjects after accounting for systematic individual change. For an example of this approach relating cognitive and brain imaging measures see [135].

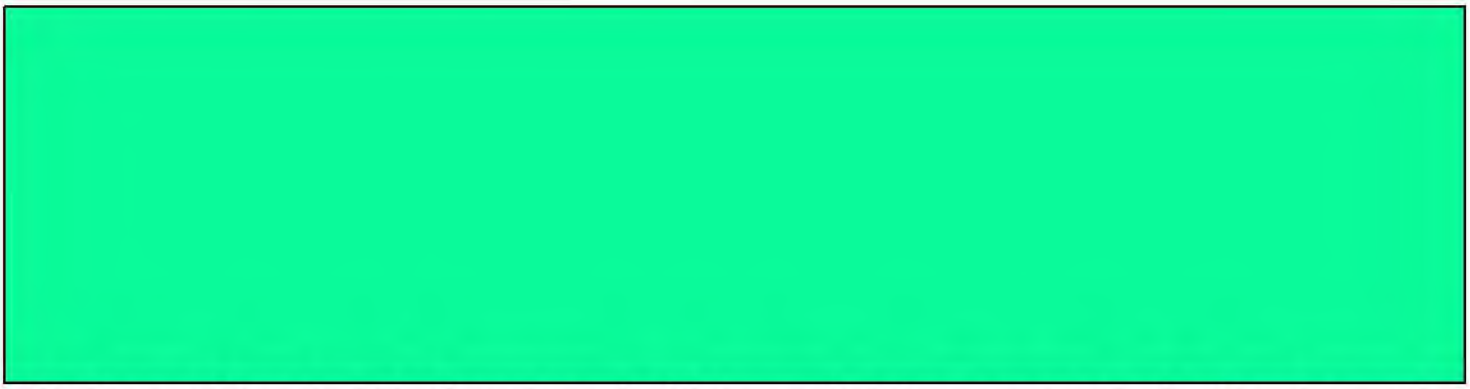
Potential pitfalls and contingencies. Although anesthesia may affect functional activity, we previously characterized functional networks [75,76], and observed that the DMN appeared similar to humans (Fig. 8), in NHPs under isoflurane anesthesia. Anesthesia will not affect results of the structural (T1-weighted and DKI) modalities so this concern will not apply to information on the anatomic profile of chronic EtOH effects. Although unexpected, treatments that effectively reduce EtOH drinking may not alter functional connectivity in the mPFC. If this occurs, several secondary analyses can be performed to shift focus to regions that may be affected based on the pharmacology of the compound in question.

C.2b. Procedures.

C.2b.1. Social rank determination. 12 adult male cynomolgus monkeys will be group-housed [redacted] in cages that permit the insertion of partitions to separate monkeys into quadrants of the cage for EtOH drinking sessions and feeding. Social groups will be constructed and ranks determined as described previously [85,86]. Socialization will begin by pair-housing each monkey in the pen with each of the other 3 monkeys for 3 days. On the first day of group housing, partitions will be removed and interactions will be monitored carefully. During the first week monkeys will be individually housed overnight for their safety. After one week they will remain in social groups 24 hrs/day. From week 2-12, two 15-min observation sessions will occur will occur for each pen each week. Social interactions will be recorded from each group using a video camera; behavior will be scored from the video using Noldus software. Individual social behaviors will be categorized as aggressive, submissive, affiliative or sexual. For each interaction both the initiator and the recipient will be recorded. Determination of ranks is based on the outcome of agonistic interactions [136]. The animal in each group that defeats all others will be designated the #1-ranked monkey. The monkey that defeats all but the #1-ranked monkey will be designated #2-ranked and so on. [redacted] The dominance hierarchy will be assessed weekly by observing social interactions when treats are placed within the pen.

C.2b.2. EtOH self-administration.

[redacted]



C.2c. Rigor and Reproducibility. Several aspects of the research strategy ensure a robust, unbiased approach:

- the proposed self-administration model has been used for many years [e.g. 38]; there is a great deal of data against which to compare the present results
- experiments are properly powered for both behavioral and imaging analyses; the Center has a dedicated statistical consultant with whom we will consult
- observational and imaging analyses will be performed by individuals blinded to the monkeys' social ranks
- the longitudinal design in which each monkey serves as its own control reduces the extent to which inter-subject variability can confound results.
- To reduce measurement-based variability all imaging will occur on the same scanner which undergoes routine quality assurance assessments.

C.3. Limitations and Future Directions

- Sex differences exist in AUD as well as the proposed EtOH drinking model [38]. Although studies with NHP subjects are exempt from the NIH's statement of expectation that both sexes be studied (i.e. NOT-OD-15-102) conducting parallel studies in females is critical to fully translate our findings. Considering the setting of this project within a budget-capped NIAAA P50 Center and the necessity of social groups of 4 subjects/pen, inclusion of females was not feasible. We plan to pursue extramural funding through other mechanisms (R01) to conduct these important studies.
- There is some concern that anesthesia may affect the BOLD signal, limiting translation of functional studies. However, functional networks can be identified under anesthesia ([75,76,111]; Fig. 8). Comparison of our NHP data to those of Project 1 will provide an empirical determination of the extent of influence of anesthesia on these measures. Moreover, even if there are some differences in magnitude of effects, our data will provide qualitative information to help focus human studies to specific ROIs. Moreover, all animals will be scanned under the same conditions. Finally, we note that we have begun to train animals for awake imaging, which will obviate the future use of anesthesia.
- The lack of a control group not exposed to EtOH could be viewed as a limitation. To mitigate this concerns, we note that all monkeys will be adults and will be treated identically within each experimental group.
- Monkeys drink identical amounts of EtOH during the induction procedure (C.2b.2); the inability to assess differential consumption during this earliest period of EtOH self-administration may be seen as a limitation. This concern is mitigated, however, by the predictive utility of drinking topography (see Expt. 1.1).
- In the future, we plan to interact with the Monkey Alcohol Tissue Research Resource, a NIAAA-funded R24 tissue resource of which Dr. Daunais is Co-PI. Stored brains from previous NHP cohorts exposed to the identical drinking protocol will be used to collaborate with WF-TARC investigators. For example, a pilot study with Project 4 in underway to assess effects of EtOH drinking on mTOR function in the NHP BLA.

(D) Interactions With Other WF-TARC Projects

The approach described above was designed to foster interactions and translation with the other WF-TARC projects. Overlapping elements of the design and opportunities for integration and translation include:

- the vulnerable phenotype generated in NHP is based on social experience (like P4)
- Specific Aim 1 will examine EtOH drinking as a dependent variable (like P3, P4)
- Expt. 1.3 will examine EtOH seeking, a behavioral measure of craving that parallel studies in P1
- choice of test drugs will be based on results in rodent (P3,P4) and, possibly, human imaging studies (P1)
- Specific Aim 3 will consider EtOH intake as an independent variable (like P1)
- Specific Aim 3 uses a set of imaging modalities that overlap those that will be used in humans (P1)

VERTEBRATE ANIMALS

1. Proposed use of animals. All proposed procedures will be performed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (2011) and have been previously approved as described in this grant application by the Wake Forest University Animal Care and Use Committee.

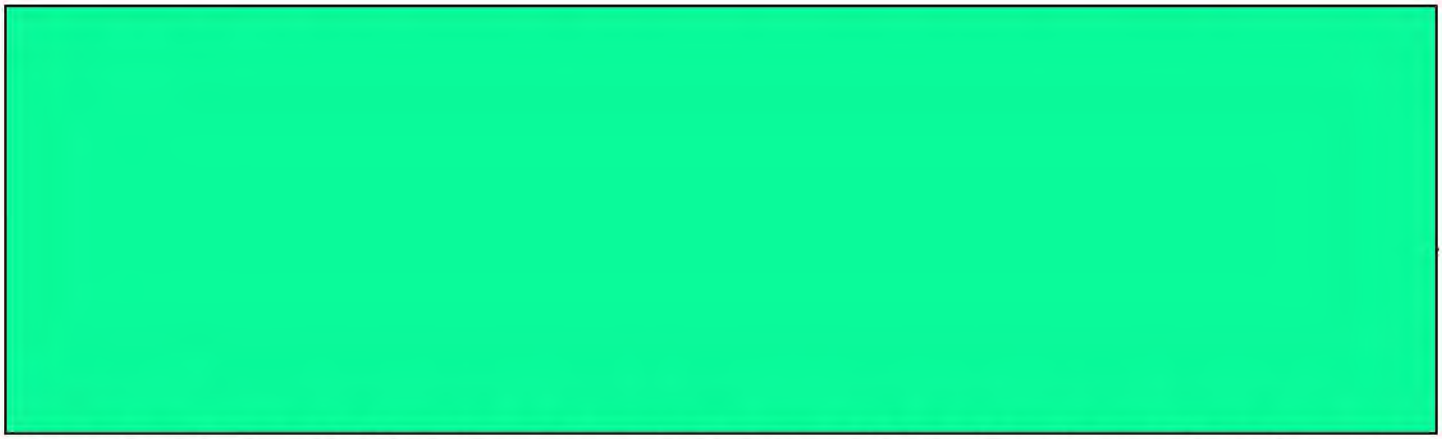
1a. Subjects and housing: Either 18 or 20 adult cynomolgus monkeys (*Macaca fascicularis*) will serve as subjects. Each subject will be weighed weekly and fed enough food (LabDiet Monkey Chow [PMI Nutrition International, LLC, Brentwood, MO), fresh fruit and enrichment items) to maintain body weights at a healthy target weight that is set to prevent the monkey from becoming obese. Target weights will be increased over time as the monkey grows as long as he remains healthy, as determined by daily inspection by laboratory personnel and periodic veterinary exams. Monkeys will be housed in groups of 4 monkeys in cages (0.75 x 1.75 x 1.80; Allentown Caging, Allentown, PA) that can be separated into quadrants with removable wire-mesh partitions when monkeys need to be singly housed for feeding and for EtOH self-administration experiments. Water will be continuously available from a spout in each quadrant of the cage. To facilitate handling, monkeys will wear aluminum collars (Primate Products, Redwood City, CA) and will be trained to approach the front of the cage when the investigator is present.

1b. Introduction of social housing conditions: Cage partitions will remain in place while monkeys are in quarantine and during the initial phase of the study while baseline MRI scans are conducted. Monkeys will, however, have visual, auditory and limited tactile contact with each future pen-mate. The 12 monkeys will be randomly assigned to three pens by weight, with the three heaviest monkeys designated to three separate pens, the next three heaviest randomly placed into those pens, and so on. Next, monkeys will be pair-housed with each other future pen-mate for 3 days each. When all pairs are deemed compatible, group housing will commence. At approximately 9:00am on the first day of social housing, all animals in the pen will receive a low, minimally sedating dose of ketamine (1.0–3.0 mg/kg). Next, the two vertical partitions and one horizontal partition will be removed. For the next 5 days, partitions will be replaced at approximately 5:00pm and monkeys will remain separated overnight. This precaution will be taken to prevent injury occurring when laboratory personnel are not present. When monkeys are separated overnight, visual, olfactory and auditory interactions will be maintained, as monkeys remain in close proximity. After approximately 1 week, monkeys will be socially housed overnight and separated at 11:00 am to eat. In rare cases of an injury that requires veterinary care or other routine veterinary procedures, the entire pen will be individually housed overnight (or until the injury heals).

1c. Determination of social ranks: From week 2 to week 12 of social housing, two focal observation periods will occur for each pen. Each 15-min session will began immediately after partitions are removed after feeding. A video camera set on a tripod will record the session, with no personnel present in the room during recording. Aggressive, submissive and affiliative behaviours will be scored according to an ethogram described previously (Morgan et al. 2000) using Noldus Observer software (Noldus Information Technology; Wageningen, The Netherlands). In these focal group sessions, both initiators and recipients of behaviours will be recorded. The monkey in each pen aggressing towards all other monkeys and submitting to none will be ranked no. 1 (most dominant). The monkey aggressing at everyone except the no. 1-ranked monkey and submitting only to the no. 1-ranked monkey will be ranked no. 2, and so on. In our laboratory's 20 years of experience conducting such studies, the monkey designated no. 4 typically displays a low frequency of aggressive behaviors and submits to all other monkeys in the pen. Thus, a transitive, linear hierarchy is established in each pen.

1d. Ethanol self-administration apparatus. Procedures for inducing and maintaining ethanol (EtOH) self-administration will be identical to those published previously (Vivian et al., 2001). These will occur in the home cage once it has been partitioned into quadrants and monkeys are individually housed. Each quadrant will contain a drinking panel (pictured in Vivian et al. 2001) containing two drinking spouts, a set of three 2.8-W lights (red, amber, and green) above each spout, one retractable lever below one of the spouts and a 2.8-W white light and a dowel within a centrally positioned opening. An active panel will be signaled by illumination of the amber lights; correct placement of the hand and pulling the dowel will be signaled by illumination of the white and green lights. Illumination of the green lights will indicate that fluid and food are available. The red lights signal a fluid flow of approximately 1.5 ml/sec. Each drinking panel will be connected

to individual EtOH and water reservoirs (2-liter bottles), positioned atop balances that continuously monitor weight displacement that is subsequently converted to volume displacement. Correct placement of the hand and pulling the dowel will operate solenoid valves that allow the fluids to become available. Fluid flow via gravity is accomplished when the EtOH or water spouts are displaced approximately 2 mm in any direction, and greater volumes were delivered when negative pressure is also applied. Thus, the volume and rate of EtOH or water consumed are determined solely by the monkey. When the monkey fails to displace the spout or removes his hand from the dowel, fluid flow will stop. Control of experimental events and data collection are performed via a National Instruments (National Instruments, Austin, TX) interface connected to a personal computer.



1f. Feeding. On days of EtOH drinking sessions, food will be available in 3 "meals." In each meal, a monkeys will earn 1/3 of his daily food allotment by responding on the lever. The first meal will start at the beginning of the drinking session (11:00 a.m.), with the other meals beginning 2 and 4 hours later. On days on which monkeys do not partake in an EtOH drinking session, they will be individually housed for two hours during which the day's allotment will be consumed. Drinking sessions will last 22 hours. Each day at 9:00a.m. (when the previous day's session ends), technical staff will enter the room, refill the feeders and fluid reservoirs, download data and restart the session at 11:00 a.m.

1g. Blood Ethanol Concentrations (BECs): Monkeys will be trained to extend their leg through a hole in the cage to permit collection of blood samples for BEC analysis. This procedure involves brief restraint and minimal discomfort. BEC will be measured every week throughout the study in order to correlate BEC with daily EtOH intake. Blood (20 microliters) will be collected from the saphenous vein and samples will be sealed in air-tight headspace vials containing 500 microliters of distilled water and 20 microliters of isopropanol (200%; internal standard), and stored at -20°C until assayed by gas chromatography (Agilent 78990N; Agilent Technologies, USA) with a headspace autosampler, flame ionization detector, and a GC Chemstation integrator.

1h. General imaging procedures. Brain imaging studies (MRI), which will last approximately 3 hours while the monkey is anesthetized, will be conducted several times over the course of this research. Approximately 20 minutes before the MRI, the monkey will be anesthetized with 10 mg/kg ketamine and transported to the imaging facility. When the monkey arrives at the MRI Center, he will be intubated and anesthesia will be maintained by 1.5% isoflurane. During the scan, the monkey's temperature will be maintained through the use of a heating pad. The following vital signs will be monitored throughout the scanning procedure: heart rate, blood pressure, respiration and body temperature. In addition, lactated ringers will be administered i.v. at 5 ml/kg/hr, for fluid maintenance. Following each scan, the monkey is returned to his home cage and monitored by laboratory staff until he is fully recovered.

Structural imaging will include: T1-weighted 3D MPRAGE (TR=2700 msec; TE=3.39 msec; TI=880 msec; FA=8 degrees; 160 slices, voxel dimension = 0.5 x 0.5 x 0.5 mm), 3D T2 FLAIR (TR=6000ms; TE=283ms; TI=2200ms, 120 slices, voxel dimension = 0.95 x 0.95 x 0.95 mm), and diffusion imaging (TR=2600 msec;

TE=110 msec; 36 slices, voxel dimension = 1.9 x 1.9 x 1.9 mm; parallel imaging factor = 4, multi-band factor = 2, 10 b0 images; 30 directions for each b value of 1000, 2000, and 3000) for measuring diffusion tensor, kurtosis, and neurite density parameters.

Functional imaging will include arterial spin labeling (MP-PCASL: labeling duration=1600 ms; post-labeling delay=1200 ms; TR= 3500ms; 20 slices, voxel dimension = 2.8 x 2.8 x 3 mm; 8 ASL phases, 15 averages) for measuring quantitative cerebral blood flow, and resting-state BOLD fMRI (TR=700 ms; TE=32 ms; 34 slices, voxel dimension=1.5 x 1.5 x 1.5 mm; multi-band factor=4) for measuring functional connectivity.

1i. Consideration of relevant biological variables. These studies will be conducted in young adult male cynomolgus monkeys who will be obtained from a single same vendor. We acknowledge that studying one sex could be viewed as a limitation. However, to ensure that the study is properly powered to detect differences between different sexes would require doubling the number of subjects. Considering the fixed budget of P50 Centers, it is not possible to accomplish this in the present proposal. We note that the NIH's recent statement of expectation that males and females both be included in research designs (NOT-OD-15-102) exempt nonhuman primates. However, there are clear sex differences observed both clinically (in AUD) and preclinically (in patterns of long-term EtOH consumption in monkeys) that make this an critically important question. Our dedication to this issue is reflected in the history of all the Investigators on this project of studying sex differences. We plan to submit additional extramural applications via mechanisms (e.g., R01) that permit larger budgets that can accommodate sufficient numbers of both males and females.

2. Rationale for involving animals and appropriateness of the species and numbers. Alcohol abuse is clearly a significant public health problem. An important aspect of making rational decisions regarding our approach to drug abuse is to collect scientific information regarding long-term effects of alcohol use on the brain and behavior. This grant application is designed with that aim in mind. There are several reasons for using nonhuman primates in these studies. Macaques have been used in drug self-administration research for over 50 years and have proven to be a valid and reliable model of human alcohol abuse. Thus there is a large database of scientific information upon which to draw and with which to integrate our results. In addition, monkeys are more genetically, phylogenetically, anatomically and physiologically similar to humans than are other research animals, enhancing our ability to accurately translate findings to human alcoholics. Moreover, monkeys are capable of learning the complex behavioral procedures necessary to serve as a model of human drug abuse. Particularly relevant to the present application, monkeys have an extensive social repertoire, forming linear dominance hierarchies that serve as a model of chronic social stress. We have extensive experience with this species studying ethanol self-administration and performing brain imaging experiments. Enhancing the translational value of these experiments, the proposed experiments have been designed to complement and extend projects in rodents and humans within the Center. For example, drugs found to be successful in reducing EtOH drinking in rodents will be tested in our more sophisticated nonhuman primate models. The imaging techniques that will be used in monkeys likewise use the same imaging modalities and equipment that will be used in humans in Project 1.

Determining the number of monkeys to use involves a trade-off between the expense of using a large number of valuable animals and confidence in the reliability of data collected using a small number of animals. The proposed experiments are designed to increase the reliability of data from small numbers of animals by using a within-subjects design, in which an animal serves as its own control. Power calculations based on published (e.g., Nader et al., 2006) and preliminary ethanol drinking data indicate that a minimum of five monkeys will be needed to reliably assess statistically significant differences between the groups at the same parameters. Because monkeys will be housed in groups of 4 monkeys per pen (2 dominant, 2 subordinate), the n=6 for our group-based analyses.

3. Veterinary care. The research will be conducted with veterinary supervision from the Animal Resources Program of Wake Forest University Health Sciences (WFUHS), headed by the attending veterinarian, [redacted] who has over 15 years of experience dealing with the subjects and methods involved in the proposed research. WFUHS is AAALAC accredited and has an approved Assurance from the NIH Office of Protection from Research Risks (OPRR). The Assurance number is A-3391-01. In addition, Wake Forest University has an Environmental Enrichment Coordinator in the Animal Resources Program who works closely with laboratory staff to assure that each animal's psychological well-being is monitored and

enriched to the greatest degree possible. Each nonhuman primate ACUC protocol has an accompanying SOP for the Psychological Well-Being of Primates. As part of this SOP, laboratory technicians maintain a log book noting daily enrichment, both behavioral and nutritional, for each monkey. This log book is examined weekly by ARP veterinary staff.

4. Minimizing pain and distress. Discomfort will be limited to what is unavoidable in conducting the research. Analgesic, anesthetic and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain, e.g. for catheter implantation. The research will be conducted with veterinary supervision from the Animal Resources Program of Wake Forest University Health Sciences, an institution accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. As described above, vital signs will be carefully monitored during anesthesia, and animals will be closely observed after anesthesia is discontinued.

5. Euthanasia. The proposed studies do not involve any terminal procedures. However, in the extremely unlikely event that unplanned euthanasia is found to be necessary, an overdose of sodium pentobarbital (100 mg/kg, i.v.) will be used. This method is consistent with the *Euthanasia of the American Veterinary Medical Association Guidelines on Euthanasia*.

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Resource Sharing Plan

Information about our research will be made freely available to the scientific and public communities via presentations at scientific meetings and invited seminars. Completed data sets will be published in a timely manner, and we will submit our accepted manuscripts to PubMed Central which allows free public access. We will make any resources that are developed as a result of this application available to other researchers. For example, we plan to interact closely with the Monkey Alcohol Tissue Research Resource (www.matrr.com), a NIAAA funded R24 tissue resource of which [redacted] is Co-PI.

AUTHENTICATION OF KEY BIOLOGICAL/CHEMICAL RESOURCES

Drugs to be used in these experiments will be procured from well-established, thoroughly validated commercial suppliers who will authenticate them prior to receipt. Whenever possible, we will use the same commercial sources as will be used by other Projects in this Center.