RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF MENTAL HEALTH

Federal Award Date: 01/30/2017

Grant Number: 2R01MH046729-23
FAIN: R01MH046729

Principal Investigator(s):
Ned H Kalin, MD

Project Title: Development and Regulation of Emotion in Primates

Brenda A Egan
Managing Officer
21 N. Park Street, Suite 6401
MADISON, WI 537151218

Award e-mailed to: NIH@rsp.wisc.edu

Period Of Performance:
Budget Period: 04/01/2017 – 01/31/2018
Project Period: 07/01/1990 – 01/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $782,066 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF WISCONSIN-MADISON 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 Of Mental Health of the National Institutes of Health under Award Number R01MH046729. 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,

Maggie C. Paolini
Grants Management Officer
NATIONAL INSTITUTE OF MENTAL HEALTH

Additional information follows

SECTION I – AWARD DATA – 2R01MH046729-23

Award Calculation (U.S. Dollars)

Salaries and Wages	$57,528
Fringe Benefits	$17,826
Personnel Costs (Subtotal)	$75,354
Materials & Supplies	$15,350
Other	$384,094
Subawards/Consortium/Contractual Costs	$42,375
Federal Direct Costs	$517,173
Federal F&A Costs	$264,893
Approved Budget	$782,066
Total Amount of Federal Funds Obligated (Federal Share)	$782,066
TOTAL FEDERAL AWARD AMOUNT	$782,066
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$782,066

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
23	$782,066	$782,066
24	$692,000	$692,000
25	$688,345	$688,345
26	$701,560	$701,560
27	$706,252	$706,252

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

Fiscal Information:

CFDA Name:	Mental Health Research Grants
CFDA Number:	93.242
EIN:	1396006492A1
Document Number:	RMH046729G
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017	2018	2019	2020	2021
MH	8022557	$782,066	$692,000	$688,345	$701,560	$706,252

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

NIH Administrative Data:
PCC: 72-NBA / **OC**: 414B / **Released**: [redacted] 01/23/2017
Award Processed: 01/30/2017 12:12:58 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01MH046729-23

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 – 2R01MH046729-23

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

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

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

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

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

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

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

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

This award has been assigned the Federal Award Identification Number (FAIN) R01MH046729. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

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

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

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

Treatment of Program Income:

SECTION IV – MH Special Terms and Conditions – 2R01MH046729-23

AWARD NOTICE:
This award has been made in response to the application submitted under the Funding Opportunity Announcement PA13-302, which can be referenced at: http://grants.nih.gov/grants/guide/pa-files/PA-13-302.html.

ADMINISTRATIVE REDUCTION:
In order to meet Institute program objectives within Fiscal Year 2017 budget constraints, future year recommended levels of support for this grant have been reduced by 10%.

BUDGET/PROJECT PERIOD ADJUSTMENT:
This grant has been selected under the NIMH plan to redistribute grant workloads more evenly throughout the year. Consequently, the initial budget period reflects a 1/31/2018 end date. Subsequent budget periods will begin on 2/1, and will be for a 12 month duration. Although this grant will have a slightly shorter budget period this year, it is awarded the full 12 month level of funds for the budget period. If needed, additional time may be requested at the end of the project period for a first no-cost extension through eRA Commons.

CONSORTIUM/CONTRACTUAL COSTS:
This award includes funds for consortium activity with the **Icahn School of Medicine at Mount Sinai**. Each consortium is to be established and administered in accordance with the NIH Grants Policy Statement, (November 2016) http://grants.nih.gov/grants/policy/nihgps/index.htm. No foreign performance site may be added to this project without the written prior approval of the National Institute of Mental Health.

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: Michael Fratina
Email: michael.fratina2@nih.gov **Phone**: 301-443-0645

Program Official: Janine M Simmons
Email: simmonsj@mail.nih.gov **Phone**: 301-443-6652 **Fax**: 301-402-4740

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01MH046729-23

INSTITUTION: UNIVERSITY OF WISCONSIN-MADISON

Budget	Year 23	Year 24	Year 25	Year 26	Year 27
Salaries and Wages	$57,528	$49,975	$74,476	$63,901	$135,333
Fringe Benefits	$17,826	$16,000	$24,444	$23,334	$49,765
Personnel Costs (Subtotal)	$75,354	$65,975	$98,920	$87,235	$185,098
Materials & Supplies	$15,350	$4,590	$18,180	$5,940	$6,840
Travel			$1,350		$2,700
Other	$384,094	$356,796	$329,649	$325,478	$130,070
Subawards/Consortium/Contractual Costs	$42,375	$38,138		$47,771	$192,068
Publication Costs			$1,800		$2,700
TOTAL FEDERAL DC	$517,173	$465,499	$449,899	$466,424	$519,476
TOTAL FEDERAL F&A	$264,893	$226,501	$238,446	$235,136	$186,776

TOTAL COST	$782,066	$692,000	$688,345	$701,560	$706,252

Facilities and Administrative Costs	Year 23	Year 24	Year 25	Year 26	Year 27
F&A Cost Rate 1	53%	53%	53%	53%	53%
F&A Cost Base 1	$499,798	$427,361	$449,899	$443,653	$352,408
F&A Costs 1	$264,893	$226,501	$238,446	$235,136	$186,776

PI: **Kalin, Ned H**	Title: Development and Regulation of Emotion in Primates	
Received: 03/07/2016	FOA: PA13-302	Council: 10/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
2 R01 MH046729-23	Dual: HD	Accession Number: 3917393
IPF: 578503	Organization: UNIVERSITY OF WISCONSIN-MADISON	
Former Number:	Department: PSYCHIATRY	
IRG/SRG: PMDA	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 23: 499,798 Year 24: 499,846 Year 25: 499,885 Year 26: 499,633 Year 27: 499,318	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
NED KALIN MD	The Board of Regents of the University of Wisconsin System	PD/PI
[redacted] name/identifier	The Board of Regents of the University of Wisconsin System	Co-Investigator
	The Board of Regents of the University of Wisconsin System	Co-Investigator
	The Board of Regents of the University of Wisconsin System	Co-Investigator
	University of Southern California	Other (Specify)-Consortium PI
	Mount Sinai School of Medicine	Other (Specify)-Consortium PI
	University of Rochester	Other (Specify)-Consortium PI
	The Board of Regents of the University of Wisconsin System	Other (Specify)-OSC
	The Board of Regents of the University of Wisconsin System	Other (Specify)-OSC

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
MH046729

b. Agency Routing Number

2. DATE SUBMITTED
2016-03-07

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 161202122

Legal Name*: The Board of Regents of the University of Wisconsin System
Department:
Division:
Street1*: Suite 6401
Street2: 21 N Park St
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53715-1218

Person to be contacted on matters involving this application

Prefix: First Name*: DEBORAH Middle Name: M Last Name*: MELTZER Suffix:
Position/Title: Assistant Dean
Street1*: 750 HIGHLAND AVE
Street2: 4115 HLTH SCI LEARNING CTR
City*: MADISON
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53705-2221
Phone Number*: 6082634940 Fax Number: 6082650522 Email: DMELTZER@WISC.EDU

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 396006492

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

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

8. TYPE OF APPLICATION*

❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

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

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Development and Regulation of Emotion in Primates

12. PROPOSED PROJECT

Start Date* Ending Date*
09/01/2016 08/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
WI-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: NED Middle Name: H Last Name*: KALIN Suffix: MD

Position/Title: DEPT CHAIRPERSON
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: PSYCHIATRY
Division: Medicine and Public Health
Street1*: 6001 RESEARCH PARK BLVD
Street2: UW PSYCH INST & CLINIC
City*: MADISON
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53719-1176
Phone Number*: 6082636079 Fax Number: 6082639340 Email*: NKALIN@WISC.EDU

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,880,144.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,880,144.00
d. Estimated Program Income*	$0.00

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

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

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: BRENDA Middle Name: A Last Name*: EGAN Suffix:

Position/Title*: Managing Officer
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Research & Sponsored Programs
Division:
Street1*: 21 N. Park Street, Suite 6401
Street2:
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53715-1218
Phone Number*: 608-262-3822 Fax Number: Email*: preaward@rsp.wisc.edu

Signature of Authorized Representative*
BRENDA A EGAN

Date Signed*
03/07/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_letter1024276502.pdf

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name:	The Board of Regents of the University of Wisconsin System
Duns Number:	161202122
Street1*:	Suite 6401
Street2:	21 N Park St
City*:	Madison
County:	Dane
State*:	WI: Wisconsin
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	53715-1218
Project/Performance Site Congressional District*:	WI-002

Project/Performance Site Location 1

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

Organization Name:	University of Southern California
DUNS Number:	072933393
Street1*:	Department of Contracts and Grants
Street2:	2001 N Soto Street, SSB 205
City*:	Los Angeles
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-9235
Project/Performance Site Congressional District*:	CA-034

Project/Performance Site Location 2

❍ 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:	Icahn School of Medicine at Mount Sinai
DUNS Number:	078861598
Street1*:	One Gustave L Levy Place, Box 1075
Street2:	
City*:	New York
County:	
State*:	NY: New York
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	10029-6574

Project/Performance Site Congressional District*: NY-013

Project/Performance Site Location 3

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

Organization Name: University of Rochester
DUNS Number: 041294109
Street1*: 518 Hylan Building
Street2: 601 Elmwood Ave
City*: Rochester
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 14627-0140
Project/Performance Site Congressional District*: NY-028

File Name

Additional Location(s)

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A3368-01

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	EMOTIONS_GRANT_SUMMARY1024225549.pdf
8. Project Narrative*	Project_narative_EMOTIONS___March_20161024276258.pdf
9. Bibliography & References Cited	Bibliography_Final1024276438.pdf
10.Facilities & Other Resources	FACILITIES_EMOTIONS_20161024225551.pdf
11.Equipment	EQUIPMENT_EMOTIONS__20161024276257.pdf
12. Other Attachments	Authentication_of_Key_Resources_Plan1024276505.pdf

PROJECT SUMMARY

Anxiety disorders can be severely debilitating, are prevalent in females, and represent a significant public health burden. These disorders often begin with early-life dispositional anxiety that can lead to the development of anxiety and depressive disorders and co-morbid substance abuse. New treatment strategies aimed at early-life anxiety are needed and have the potential to prevent this life-long suffering. The nonhuman primate (NHP) model of early-life anxious temperament (AT) is ideal because of similarities between rhesus monkeys and humans in the development of socio-emotional behavior and its underlying neural circuits. Our work, and that of others, strongly implicates altered function of the extended amygdala as a core feature of AT and anxiety disorders. The extended amygdala includes the central nucleus of the amygdala (Ce) and the bed nucleus of the stria terminalis (BST). The extended amygdala integrates threat-relevant information from cortical and subcortical inputs, and initiates behavioral and physiological responses to threat. Of particular interest are the orbitofrontal “regulatory” influences on extended amygdala function and anxiety. In considering the development of new treatments, there are a number of critical questions. Advances in molecular technologies for reversibly and bi-directionally controlling brain function are beginning to make some of these questions tractable. Designer receptors exclusively activated by designer drugs (DREADDs) are ideal for examining early-life AT in NHPs because they can modulate critical brain regions for long periods of time (i.e. hours) -- particularly relevant for uncovering mechanisms related to mood and anxiety dysregulation. The DREADDs technique involves infecting a brain region with a viral vector that expresses a receptor that does not naturally occur in the brain, which is then combined with a pharmacological intervention, an otherwise “inert” drug that selectively activates DREADDs. Importantly, DREADDs can be used to chronically alter circuit function to model long-term brain alterations associated with psychopathology. We established a Cre-Lox recombination strategy to express DREADDs in NHP amygdala neurons that project to select effector sites. This allows unprecedented control of specific projections in the brains of freely behaving NHPs during exposure to ethologically relevant contexts. This proposal will use DREADDs in young female NHPs to understand how, early in life, projections in the extended amygdala drive sustained anxiety-related behavior and how this circuit is modulated by direct projections from caudal orbitofrontal cortex. It will also explore whether chronic early-life activation of the Ce is sufficient to induce extreme anxiety accompanied by the functional and structural brain changes associated with stress-related psychopathology. Lastly, the proposed studies will identify molecular markers of projection-specific anxiety-modulating neurons that will enable development of selective circuit-based treatment approaches. Together these studies will set the stage for the feasibility of amygdala projection-specific treatments for early-life anxiety, and demonstrate the utility of an animal model to test these novel approaches.

PROJECT NARRATIVE

An extreme and stable anxious temperament (AT) during childhood is a significant risk factor for the later development of anxiety disorders, especially in adolescent girls. Using our non-human primate (NHP) model of AT, the proposed studies will use multimodal neuroimaging and advanced molecular genetic techniques to identify and selectively manipulate the activity of neurons in specific projections within the AT-related neural circuit in freely behaving young female NHPs. The work in this proposal will also identify novel molecular targets for drug discovery with the potential to prevent the consequences of chronic early life anxiety.

FACILITIES AND OTHER RESOURCES

Scientific Environment:
The University of Wisconsin has a long and recognized history of research with nonhuman primates, as well as the study of the neurobiology of emotion. The scientists involved in this endeavor are part of the Departments of Psychiatry, the HealthEmotions Research Institute (HERI) and Lane Neuroimaging Laboratory, the Wisconsin National Primate Research Center, the Harlow Laboratory for Biological Psychology, the Wisconsin Institute for Medical Research (WIMR), and the [redacted]. In addition to being affiliated with these institutions, this research team also plays a leadership role in them. This means that institutional support for neuroscience research and basic science research is extremely strong. Our laboratory is investigating the neurobiological basis of fear, anxiety, and depression at preclinical and clinical levels. One of the strengths of our approach is that we are working across a variety of technologies (molecular, preclinical animal models including primates, and human functional brain imaging) to maximize our ability to understand the neural circuitry underlying normal as well as pathological emotional states. This unique research team draws on the strong individual, but interconnecting expertise of each scientist, which is a factor that greatly contributes to the probability of successfully implementing the Aims of this project. As evidenced by their long publication history there is a close collaborative relationship and intellectual rapport among [redacted]

All Facilities and equipment listed below are located in close proximity and available for use in this project.

Laboratory:
The UW Department of Psychiatry [redacted] sq. ft.) dedicated to developmental neurobiology, analytical biochemistry, and molecular biology. All the necessary equipment for radioimmunoassay, HPLC, ELISAs, and other standard laboratory procedures is available in [redacted]. A cold room, microscopy room containing several microscopes including an inverted fluorescent microscope, cell culture room with CO_2 incubators, instrument rooms equipped with a gamma counter for counting ^{125}I, autoclaving, sample storage, and dishwashing facilities are on site. The cortisol and corticotropin-releasing hormone assays will be performed at this facility.

Radiochemistry Laboratory:
The [redacted], radiochemistry labs, research PET scanner, electronics development labs, and smaller rooms for HPLC, GC, and other chemical analyses ([redacted].). An adjacent, fully equipped radiopharmaceutical facility assists in the synthesis and analysis of numerous PET agents. The lab is equipped with 3 shielded fume hoods, 8 HPLCs, 2 GCs, Ge and NaI spectrometers, Capintec and high sensitivity Xe filled well counters, roto-evaporators, vacuum pumps, and numerous modular radiation detectors and electronics. This facility houses an accelerator, PET and MRI scanners and support facilities for wet labs, radiochemistry, data analysis, and instrument fabrication. The imaging facility is supported by a large group of scientists and technicians from the Departments of Medical Physics and Radiology, with whom the PI has extensive collaborative contacts. Radioligand preparation and image analysis will be conducted at these sites.

Animal:
Approximately 2,000 rhesus macaques are housed in colonies at the [redacted]
These facilities are fully accredited and have [redacted] sq. ft. containing animal housing, squeeze cages, transport cages, test facilities, data acquisition and control hardware and software, fully equipped operative suites which include anesthesia machines, autoclave, surgical instruments, and monitoring equipment, neuropathology, histology and general pathology laboratories, and an extensive library. Twenty-four hour veterinary care is backed by a clinical laboratory and provided as part of the daily animal care charge. Preparatory procedures and recovery will be accommodated by the extensive facilities, encompassing five buildings on the main UW campus. These facilities will be used for animal housing, behavioral testing, behavioral data analysis, office space, PET scanning, surgery, sample preparation, and storage.

MRI Facilities:
The [redacted] consist of [redacted] sq. ft. of state of the art laboratory and office space and are located on the [redacted] to the Department of Psychiatry at the University of Wisconsin - Madison. These facilities are equipped with a G.E. 3T MR Scanner for structural and functional brain imaging. A back-up MRI scanner with similar capabilities is available at the [redacted] also has image processing areas with extensive computing facilities, and a conference room. Dr. Kalin directs the Neuroimaging Laboratory and can ensure that scanning times are available for non-human primates.

Computer:
[redacted] network is located in a temperature controlled secure location at the [redacted] All servers and SAN equipment are on generator-backed circuits, with UPS to provide power during cutovers. Authentication is provided by [redacted]

The authentication and file server is a Dell 2970 2U server with six 1TB SATA hard drives in a RAID5 configuration. It has 16GB of RAM and two 2.5 GHz processors with 4 processing cores each. Two Broadcom network interfaces aggregated in an 802.3ad link aggregate for 2 Gb/s of total user throughput. Connection to the SAN is through 4 Broadcom iSCSI adapters, also aggregated for 4 Gb/s of throughput. This sever will also provide alternative operating system access by way of virtualized environments using [redacted].

The application server is an Apple Xserve with two 4core Intel Xeon processors at 2.5 GHz. It has 8 Gigs of RAM and is equipped with 4 Intel network interface ports. One port is dedicated to a private network for communication with the MRI machine itself. One port will provide user access at 1 Gb/s and two 1GB/s ports proved 802.3ad aggregated access to the SAN. MRI and Simulator workstations are Dell Optiplex 760 with 2 GB RAM and 3.0 GHz processors with 4 computing cores each. Startech PCI and PCI-ex serial and parallel cards have been installed to complement the existing USB, serial, and parallel connections. They are further equipped with flash media adaptors allowing the make use of SD, MMC and other card formats for data transfer as well. Facility workstations are comprised of Apple 24-inch iMac computers with 2 GB Ram and 2.6 GHz processors. Using bootcamp, they run either OSX or Windows Vista/7. The Windows partition is further accessible using Vmware Fusion in OSX as a virtual machine. Vmware Fusion also allows access to other operating systems as necessary. Network printers include a Sharp digital MX2600N color photocopier and numerous HP LaserJet printers.

Access to a wide variety of data analysis software is available throughout the lab. Most software packages are installed on every applicable computer in the lab. The following programming languages are actively used and supported: C/C++, Java, Perl, Python, Tcl, IDL (Research Systems Inc.), and Matlab (MathWorks). Standard office-related software packages are ubiquitous, including Microsoft Office, Adobe Photoshop / Illustrator Acrobat, webpage editors, web browsers, email programs, etc. We use standard software for writing to CD/RW media. MRI: Stimulus presentation is controlled by E-Prime software (Psychology Software Tools Inc.). The fMRI data are uploaded as DICOM files and preprocessed to correct for rigid body motion and image distortion caused by magnetic field inhomogeneity. Several software packages are available for analysis of fMRI data, including SPM2/SPM5/SPM8, SnPM, AFNI, fmristat, FSL, BrainVoyager, MedX, and VoxBo. Morphometric measurements can use AIR, FSL, Freesurfer, or SPM2/SPM5/SPM8 for coregistration. Both Freesurfer and inhouse tools perform manual coregistration (BrainSqueezer) and distortion-based morphometry (DBM) measurements.

Manual ROI drawing can use AFNI or an in-house tool (BrainMaker), automated ROI identification can be performed with Freesurfer. Talairach coordinates can be investigated using the Talairach Daemon. Diffusion Weighted Imaging (DWI) uses FSL and in-house programs for data reconstruction, display, and analysis. DWI data are normalized with DTI-TK, which iteratively constructs a template from the tensor files, and tractography will be performed in Camino. Cortical flatmaps can be created using BrainVoyager. Image Display: A variety of image display programs are available, including AFNI, SPM2/SPM5/SPM8, BrainVoyager, FSL, Freesurfer, Spamalize, MedX, LORETA, BESA, and VoxBo.

Computers are networked to each other, the PET, and MRI scanners. In addition to model calculation, display, and file manipulation software written in-house, the lab uses Matlab, K.J. Friston's SPM software, R. Woods' AIR system and C. Pelizzari's co-registration programs. We also utilize AFNI from the National Institutes of Health. Several computers exist in the Cyclotron lab and the Harlow Center for Biological Psychology. These include Macs and PCs for accelerator control, HPLC, GC, and nuclear spectroscopy system. There are also Macs at the Cyclotron lab and the Harlow Center for Biological Psychology equipped with LabView software and National Instruments multipurpose data acquisition hardware for radiochemistry process monitoring and control. A CD-recorder/rewriter is used along with DAT tape for data storage.

Office:
Office space is available in the name/identifier/location

Other:
Special apparatus can be constructed on-site in the electronics and sheet metal shops at the name/identifier/ . Editorial, secretarial and photographic supports are available through the Department of Psychiatry along with pre- and post-award research administrative services.

EQUIPMENT

All equipment listed below is located in close proximity and available for use in this project.

1. Cyclotron

A negative ion 11 MeV proton cyclotron provides positron emitting radioisotopes for research PET scanners. The cyclotron, the first CTI RDS-112, is sited in a shielded bunker with adequate space for targetry development and source product telemetry. In addition, we have access to a particle accelerator (NEC 6MeV deuteron and proton tandem accelerator) for the production of short half-life tracers for PET.

2. MRI

The structural and functional MRI studies proposed in this application will be performed at the UW name/identifier/location research facility, located name/identifier/location at the University of Wisconsin - Madison. This facility is equipped with a G.E. 3T MR Scanner for structural and functional imaging. This system has multi- nuclear RF capabilities and a variety of local coils, phased array coils, receivers and specialized coils for imaging specific anatomical sites. A back-up MRI scanner with similar capabilities is available at the nearby name/identifier/location .

3. MicroPET and Accelerator

name/identifier/location is home to the above mentioned particle accelerator and a GE ADVANCE PET camera, radiochemistry labs, a simulator room and image processing areas. We have a Siemens microPET Focus 220, which is housed at the name/identifier/location and dedicated to this research. This scanner has a crystal energy resolution of 16.2 +/- 1.7%. Sensitivity at 250-750 keV and 10 ns is 12% and image resolution is 1.37 mm FWHM. The system linearity is within +/- 3% from 1 kHz to 90 kHz and attenuation and scatter corrections are accurate to 1%. Spatial Uniformity is 3.8% (0.23 mL ROIs in 10 cm phantom) and temporal Noise = 6.6% (0.16 mL ROIs, 100M counts frames, 10 cm phantom).

4. Microscope

We have a Leica LMD6500 Laser Microdissection System housed at the name/identifier/location This state-of-the-art microscope uses a laser to precisely cut out individual neurons from thin slices of tissue mounted on membrane-coated slides. Cells are collected via gravity, which decreases the risk of contamination from surrounding tissue.

5. Hormonal assay

name/identifier/location analytical biochemistry and molecular biology laboratory. The biochemistry laboratory is equipped with a UV/Vis spectrophotometer and has immunoassay capability using an automated liquid handler and plate spectrophotometer. The lab also has a gamma counter for radioimmunoassays.

AUTHENTICATION OF KEY RESOURCES

Following preparation of constructs for viral vector packaging, the identity of the insert is confirmed by sequencing. In addition, we will confirm the ability of the construct to overexpress the protein of interest through *in vitro* transfection into HEK293 cells followed by immunodetection with commercially available antibodies. After the construct is packaged into an adeno-associated virus (AAV) via a commercial vendor, we will confirm the function and identity of the overexpressed protein by infecting HEK293 cells and detecting the express protein using the same commercially available antibodies.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Personnel/Biographical sketches not requested - this page through page 61

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	NED	H	KALIN	MD	PD/PI	base salary & percent effort				9,255.00	3,424.00	12,679.00
2 .	Dr.	name			PhD	Co-Investigator					4,723.00	1,748.00	6,471.00
3 .	Dr.	name			PhD	Co-Investigator					12,000.00	4,440.00	16,440.00
4 .	Dr.	name			PhD	Co-Investigator					0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,590.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
	Graduate Students						
4	Undergraduate Students				10,000.00	240.00	10,240.00
	Secretarial/Clerical						
1	Lab Manager				8,250.00	3,053.00	11,303.00
1	Research Specialist				13,300.00	4,921.00	18,221.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**39,764.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**75,354.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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	
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**	**0.00**

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,350.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	42,375.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	128,255.00
9 . Scanning costs	65,280.00
10 . Surgery and Pathology	190,559.00
Total Other Direct Costs	**441,819.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**517,173.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	499,798.00	264,893.00
		Total Indirect Costs	**264,893.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**782,066.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: EMOTION_GRANT_RENEWAL_BUDGET_3_7_16_Final1024276515.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*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	NED	H	KALIN	MD	PD/PI	[redacted]				9,255.00	3,424.00	12,679.00
2 .	Dr.	[redacted]	[redacted]	[redacted]	PhD	Co-Investigator					4,723.00	1,748.00	6,471.00
3 .	Dr.	[redacted]	[redacted]	[redacted]	PhD	Co-Investigator					12,000.00	4,440.00	16,440.00
4 .	Dr.	[redacted]	[redacted]	[redacted]	PhD	Co-Investigator					0.00	0.00	0.00

base salary & percent effort

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **35,590.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	[redacted]					
	Graduate Students						
3	Undergraduate Students				8,000.00	192.00	8,192.00
	Secretarial/Clerical						
1	Lab Manager				8,250.00	3,053.00	11,303.00
1	Research Specialist				13,300.00	4,921.00	18,221.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**37,716.00**

percent effort

Total Salary, Wages and Fringe Benefits (A+B) **73,306.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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	
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**	**0.00**

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	5,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	42,375.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	151,920.00
9 . Scanning costs	48,960.00
10 . Surgery and Pathology	195,560.00
Total Other Direct Costs	**443,915.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**517,221.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	474,846.00	251,668.00
		Total Indirect Costs	**251,668.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**768,889.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: EMOTION_GRANT_RENEWAL_BUDGET_3_7_16_Final1024276515.pdf (Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	NED	H	KALIN	MD	PD/PI	base salary & percent effort				9,255.00	3,424.00	12,679.00
2 .	Dr.	name			PhD	Co-Investigato					9,446.00	3,495.00	12,941.00
3 .	Dr.	name			PhD	Co-Investigato					16,000.00	5,920.00	21,920.00
4 .	Dr.	name			PhD	Co-Investigato					0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **47,540.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
	Graduate Students						
4	Undergraduate Students				10,000.00	240.00	10,240.00
	Secretarial/Clerical						
1	Lab Manager				24,750.00	9,158.00	33,908.00
1	Research Specialist				13,300.00	4,921.00	18,221.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**62,369.00**

Total Salary, Wages and Fringe Benefits (A+B) **109,909.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	
Total Travel Cost	**1,500.00**

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

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	20,200.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	155,955.00
9 . Scanning costs	89,760.00
10 . Surgery and Pathology	120,561.00
Total Other Direct Costs	**388,476.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,885.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	499,885.00	264,939.00
		Total Indirect Costs	**264,939.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**764,824.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: EMOTION_GRANT_RENEWAL_BUDGET_3_7_16_Final1024276515.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 4

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	NED	H	KALIN	MD	PD/PI	base salary & percent effort				9,255.00	3,424.00	12,679.00
2 .	Dr.	name			PhD	Co-Investigator					9,446.00	3,495.00	12,941.00
3 .	Dr.	name			PhD	Co-Investigator					16,000.00	5,920.00	21,920.00
4 .	Dr.	name			PhD	Co-Investigator					0.00	0.00	0.00

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **47,540.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
	Graduate Students						
2	Undergraduate Students				1,000.00	24.00	1,024.00
	Secretarial/Clerical						
1	Lab Manager				22,000.00	8,140.00	30,140.00
1	Research Specialist				13,300.00	4,921.00	18,221.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**49,385.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**96,925.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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	
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**	**0.00**

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	6,600.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	53,078.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	153,975.00
9 . Scanning costs	103,360.00
10 . Surgery and Pathology	104,307.00
Total Other Direct Costs	**421,320.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**518,245.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	490,167.00	259,789.00
		Total Indirect Costs	**259,789.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**778,034.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: EMOTION_GRANT_RENEWAL_BUDGET_3_7_16_Final1024276515.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 5

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	NED	H	KALIN	MD	PD/PI	base salary & percent effort				9,255.00	3,424.00	12,679.00
2 .	Dr.	name			PhD	Co-Investigator					23,615.00	8,738.00	32,353.00
3 .	Dr.	name			PhD	Co-Investigator					40,000.00	14,800.00	54,800.00
4 .	Dr.	name			PhD	Co-Investigator					0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **99,832.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
	Graduate Students						
2	Undergraduate Students				1,000.00	24.00	1,024.00
	Secretarial/Clerical						
1	Lab Manager				27,500.00	10,175.00	37,675.00
1	Research Specialist				19,000.00	7,030.00	26,030.00
1	Computer Programmer				30,000.00	11,100.00	41,100.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**105,829.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**205,661.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

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

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,600.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	216,493.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	103,575.00
9 . Scanning costs	19,040.00
10 . Surgery and Pathology	21,907.00
Total Other Direct Costs	**371,615.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**580,276.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	388,783.00	206,055.00
		Total Indirect Costs	**206,055.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**786,331.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: EMOTION_GRANT_RENEWAL_BUDGET_3_7_16_Final1024276515.pdf (Only attach one file.)

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

BUDGET JUSTIFICATION

Costs are consistent with policies of the University of Wisconsin-Madison. There is no overlap between this proposal and Dr. Kalin's other grants. R01-MH081884 "Brain mechanisms mediating genetic risk for anxiety and depression" uses a combination of irreversible PFC lesions, brain imaging, and molecular genetics focused on transcriptomic alterations in the neural circuitry underlying anxiety in young male and female monkeys. Project 1 of the Conte Center P50-MH100031 grant, "Neural mechanisms mediating adversity's impact on the risk for developing anxiety" longitudinally follows infant monkeys to assess individual differences in the developmental trajectory of the onset of anxious temperament. R01-MH107563 "Extreme anxiety in females: The role of the bed nucleus of the stria terminalis (BST) during the transition to adolescence in human and nonhuman primates" studies a sample of preadolescent girls with subthreshold anxiety using imaging, clinical, and behavioral methods to examine susceptibility and the biomarkers for the risk to develop anxiety disorders. In addition, this grant includes translational nonhuman primate studies using irreversible lesions of the BST and RNA sequencing of BST neurons to understand its role in anxiety. The current proposal translates designer receptors exclusively activated by designer drugs (DREADDs) technology from rodent to NHP studies as a means to understand mechanisms relevant to the pathophysiology and treatment of stress-related psychopathology. In this proposal we combine DREADDs with methods commonly used in our lab including multimodal neuroimaging and RNA sequencing. To examine projection-specific pathways, a dual-infection Cre-Lox method will be used to selectively manipulate anxiety relevant circuitry. Additionally, we will use DREADDs to chronically activate the amygdala and directly examine the long-term effects of amygdala hyperactivation on brain structure, function and anxiety. In the last aim of this grant, RNA sequencing of viral vector-transfected neurons will allow us to identify putative molecular targets by comparing transcriptomic differences between pathway specific and non-pathway specific neuronal subpopulations.

Please note that we have strong institutional support for this proposal as evidenced by $25,000 per year in matching funds from the University of Wisconsin (UW) Psychiatry Department and $12,500 for equipment plus $62,500 for personnel from the UW Medical School (see letters of support). We also note that the subaward contract with USC for RNA sequencing is only active in budget period 5 because all of this work will be done in that year. Likewise, the subcontract with Mt. Sinai will only be in years 1 and 2, since they will be manufacturing the compound necessary for the studies. The University of Rochester subcontract will be active in years 4 and 5 since they will be analyzing tissue samples that are collected in those years.

KEY PERSONNEL

Ned H. Kalin, MD (PI) – [redacted]

Dr. Kalin will serve as Principal Investigator of this project, overseeing all related studies. He has more than 35 years of experience in primate research and will design experiments, interpret results, prepare manuscripts and coordinate interactions with key personnel and collaborators. Salary calculations are based on the $185,100/yr legislatively imposed salary cap.

[redacted] PhD (Co-I) – [redacted] in Years 1 & 2, [redacted] in Years 3 &4, [redacted] in Yr 5:

[redacted] will coordinate the daily operations of the study, including managing students and staff for imaging data collection, overseeing the positron emission tomography (PET) data analysis, including co-registration of magnetic resonance imaging (MRI) and PET images, training personnel to help with coregistration of multimodal imaging data, preliminary analyses of imaging data and integration of these measures with behavioral and physiological data. He has conducted studies using fMRI plus microPET in nonhuman primates for the past 8 years, working closely with Dr. Kalin with a focus on the role of the amygdala in emotion and anxiety in non-human primates. He has extensive experience with MRI-guided surgeries and will be responsible for infusions of viral vectors into the targeted brain regions. Due to his anatomical expertise and his experience over the past two years with LCM, he will lead the effort to collect fluorescently-labeled neurons. Lastly, he will be involved in the interpretation and publication of results in collaboration with the Dr. Kalin.

name, PhD (Co-I) – percent effort in Years 1 and 2, percent effort in Years 3 & 4, percent effort in Year 5:

name has 30 years of experience with central nervous system molecular biology and pharmacology. He will oversee the assay of plasma levels of cortisol and cerebrospinal fluid levels of corticotropin-releasing hormone (CRH). He will oversee fluorescence and immunofluorescence procedures to visualize neurons prior to LCM capture and will also supervise the work on the extraction of RNA from the LCM-captured neurons. He will assist with interpretation of the data generated from steroid and peptide hormone assays. Lastly, he will also be involved in the preparation of results for publication.

name PhD (Co-I) – percent effort cost shared effort (no salary requested):

name has several years of expertise in RNAseq data analysis and primate neuroimaging data analysis. He will be directly involved with scientists from USC to analyze the RNAseq data and will work closely with Dr. name to process and analyze the multimodal imaging data. He has conducted studies using fMRI plus microPET in nonhuman primates for the past 13 years, working closely with Dr. Kalin with a focus on the role of the amygdala in emotion and anxiety in non-human primates. He will also be involved in preparing manuscripts and disseminating research data.

KEY PERSONNEL – Other Significant Contributor with no measurable efforts

name PhD – OSC Dr. name/identifier and an expert in mapping and measuring the functional and structural organization of the human brain using MRI. In this proposed research, Dr. Alexander will serve as a consultant on diffusion tensor imaging (DTI) methods development and analysis.

name PhD – OSC Dr. B name/identifier He is an expert in functional MRI (fMRI) and functional connectivity acquisition and analysis techniques and has made important contributions to fMRI methods, especially with regard to reducing the impact of artifacts and physiological noise. Dr. Birn will serve as a Consultant in the proposed study.

name, PhD – Consultant Dr. name/identifier He is an electrophysiologist with extensive experience with patch clamp, field potential and multielectrode techniques. He will collaborate with us using electrophysiological techniques to assess DREADDs receptor function in using *in vivo* depth electrodes and *in vitro* slices of rhesus brain tissue.

name, PhD – Consultant Dr. name/identifier in the field of DREADDs receptor technology and is currently developing new receptors and ligands that will be useful in primates. He also has experience performing whole cell recordings from brain slices including non-human primate bed nucleus of stria terminalis. He will continue his collaboration with us by consulting on DREADDs experimental design and *in vivo* and *ex vivo* electrophysiological techniques for assessing DREADDs receptor function.

name, PhD – Consultant Dr. name/identifier and an expert in MRI-guided neurotherapy with a focus on guided gene delivery into the brain. Continuing an ongoing collaboration with the Kalin lab focused on the injection of viral vectors into the primate brain, name/identifier expertise for MRI guided targeting for the DREADDs studies in this proposal.

OTHER PERSONNEL

name – percent effort in Years 1 & 2, percent effort Yr 3, percent effort Yr 4 & percent effort Yr 5:

name will assist in all aspects of intubation, positioning, administration of anesthesia, and monitoring during PET and MRI scans. She will also be responsible for surgical preparation, assisting during surgery and monitoring animals during recovery. In addition, she will assist in the obtaining, processing and cataloguing of physiological samples; assignment and scheduling of subjects; and extensive record keeping, data

management, and analysis required when working with primates. Additionally, she will aid in the preparation of posters and presentations. She will be responsible for encoding behaviors during the human intruder paradigm as well as help train new students, manage records, and oversight of the budget, data reduction and analysis of behavioral and physiological data. In addition, [redacted: name] will assist with physiological sampling, training of new personnel. She will oversee the adherence to the strict regulations by providing documentation requirements unique to primate research.

Systems Programmer – [redacted: percent effort] in Year 5: This person is necessary to assist in development of the complex algorithms and programming required in this project for the analysis of large amounts of data from RNA sequencing and multimodal imaging that are generated toward the completion of the 5 year project. There is a $62,500 institutional match that will provide 60% salary and fringe support for the hire in year 5 by the School of Medicine & Public Health, with the other 40% of the salary and fringe paid for by grant funds.

Research Specialist (TBD) – [redacted: percent effort] in Years 1-4, [redacted: percent effort] in Year 5: The research specialist will assist in all aspects of intubation, positioning, administration of anesthesia, and monitoring during PET and MRI scans. They will also be responsible for surgical preparation, assisting during surgery and monitoring animals during recovery. In addition, they will assist in the obtaining, processing and cataloguing of physiological samples; assignment and scheduling of subjects; and extensive record keeping, data management, and analysis required when working with primates.

Students – [redacted: salary & percent effort]
Under the guidance of [redacted: %effort] staff students will provide assistance with general laboratory maintenance, study set up, and clean up, animal handling, and data management as well as for post procedure animal monitoring and filling out the numerous forms required as part of doing research with primates. There is additional support from undergraduate students who are assisting with the research as course credit.

While all personnel have primary duties and areas of expertise defined above, it should be noted that because of the complexities involved in working with primates using the proposed paradigms, a minimum of two and as many as four trained people are required at the same time to safely accomplish many of the procedures outlined in this proposal.

Total Personnel Cost for Entire Period: $561,154

SUPPLIES

Expenses have been determined based on the attached timeline.

Ancillary:

Costs include five years of supplies for sampling blood, tubes, pipet tips, reagents, personal protective equipment, anesthesia, instrument maintenance, gas sterilization, scanning supplies, analyses, and publication costs.

Total Ancillary Costs for Entire Period: $27,650

Viral vector production:

AIMS 1 & 2:
DREADDs
AAV5-hSyn-DIO-hM4D(Gi)-mCherry; 10^{12} – 10^{13} vg/ml; N=16
12 – 100 µl aliquots @ $220/aliquot = $2,640
AAV5-hSyn-DIO-hM3D(Gq)-mCherry; 10^{12} – 10^{13} vg/ml; N=16
12 – 100 µl aliquots @ $220/aliquot = $2,640
AAV5-hSyn-DIO-mCherry; 10^{12} – 10^{13} vg/ml; N=16
12 – 100 µl aliquots @ $220/aliquot = $2,640

CAV2-Cre
CAV2-Cre; 2 x 10^{12} pp/ml; N=48
2.5 x 10^{12} pp @ $2,800

AIM 3:
DREADDs
AAV5-hSyn-hM3D(Gq)-mCherry; 10^{12} – 10^{13} vg/ml; N=12
6 – 100 µl aliquots @ $220/aliquot = $1,320
AAV5-hSyn-EGFP; 10^{12} – 10^{13} vg/ml; N=12
6 – 100 µl aliquots @ $220/aliquot = $1,320

Total virus costs for entire period: $13,360

Assay:

Costs include standards, tubes, reagents, and radioactive tracers for measurement of plasma cortisol and CRH obtained during hormonal sampling. Costs also include reagents for immunofluorescent identification of neurons and RNA extraction of the LCM obtained samples.

AIM 1:

- Plasma cortisol:
  24 subjects with 11 samples each = 264 samples @ $4.00/sample = $1,056
- Plasma estradiol
  24 subjects with 1 sample each = 24 samples @ $6.00/sample = $144
- Plasma testosterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- Plasma progesterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- CSF/CRH Assay:
  24 subjects with 3 samples each = 72 samples @ $7.25/sample = $522
  Vet tech assistance for CSF draw for 24 subjects with 3 draws each = 72 draws @ $50/draw = $3,600

Total: $5,706

AIM 2:

- Plasma cortisol:
  24 subjects with 11 samples each = 264 samples @ $4.00/sample = $1,056
- Plasma estradiol
  24 subjects with 1 sample each = 24 samples @ $6.00/sample = $144
- Plasma testosterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- Plasma progesterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- CSF/CRH Assay:
  24 subjects with 3 samples each = 72 samples @ $7.25/sample = $522
  Vet tech assistance for CSF draw for 24 subjects with 3 draws each = 72 draws @ $50/draw = $3,600

Total: $5,706

AIM 3:

- Plasma cortisol:
  24 subjects with 11 samples each = 264 samples @ $4.00/sample = $1,056
- Plasma estradiol
  24 subjects with 1 sample each = 24 samples @ $6.00/sample = $144
- Plasma testosterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- Plasma progesterone
  24 subjects with 1 sample each = 24 samples @ $8.00/sample = $192
- CSF/CRH Assay:
  24 subjects with 7 samples each = 168 samples @ $7.25/sample = $1,218

Vet tech assistance for CSF draw for 24 subjects with 7 draws each = 168 draws @ $50/draw = $8,400

Total: $14,082

AIM 4:

- Immunohistochemistry:
  NeuN antibody: 2 vials @ $350/vial = $700
  AlexaFlour488 conjugated goat anti-mouse: 2 vials @ $185/vial = $370
- RNA sequencing:
  - RNA Extraction Kits: QIAshredder kit for cell lysis and Direct-zol RNA microprep kits for purification of total RNA including both mRNA and small RNAs ≥ 17 nucleotides
    48 subjects with 2 samples each = 96 samples
    2 QIAshredder kit = 50 samples@$85/kit = $170
    2 RNA microprep kit = 50 samples @ $400/kit = $800

Total: $2,040

Total Assay Costs for Entire Period: $27,534

Surgery:

Costs include gas anesthesia, instrument maintenance, sterilization, surgical supplies, MRI time for surgery, use of surgical suite, post-surgical observation and treatment, and veterinary and veterinary technician time.

AIM 1:

- 24 subjects with 1 surgery each = 24 surgeries @ $7,100/surgery = $170,400

AIM 2:

- 24 subjects with 1 surgery each = 24 surgeries @ $7,100/surgery = $170,400

AIM 3:

- 24 subjects with 1 surgery each = 24 surgeries @ $7,100/surgery = $170,400

Total Surgical Costs for Entire Period: $511,200

<u>OTHER COSTS:</u>

PET Scans:

Costs include preparation of radioactive tracer, technician time, and use of scanner.

AIM1:

- 24 subjects with 2 scans each = 48 scans (1 hr/scan) @ $475/hr = $22,800
- 48 doses @ $135/dose = $6,480

AIM2:

- 24 subjects with 2 scans each = 48 scans (1 hr/scan) @ $475/hr = $22,800
- 48 doses @ $135/dose = $6,480

AIM3:

- 24 subjects with 6 scans each = 144 scans (1 hr/scan) @ $475/hr = $68,400
- 144 doses @ $135/dose = $19,440

Total PET Scanning Costs for Entire Period: $146,400

MRI Scans:

Costs include the use of scanner and technician time. MRIs are necessary for co-registration of microPET data and to determine success of the surgery.

AIM1:

- 24 subjects with 2 scans each = 48 scans (1.5 hr/scan) @ $500/hr = $36,000

AIM2:

- 24 subjects with 2 scans each = 48 scans (1.5 hr/scan) @ $500/hr = $36,000

AIM3:

- 24 subjects with 6 scans each = 144 scans (1.5 hr/scan) @ $500/hr = $108,000

Total MRI Scanning Costs for Entire Period: $180,000

Pathology:

Costs include veterinarian time, use of pathology suite, sacrifice and necropsy.

AIM1:

- 24 subjects (no perfusion) @ $1,000/subject = $24,000

AIM2:

- 24 subjects (no perfusion) @ $1,000/subject = $24,000

AIM3:

- 24 subjects (with perfusion) @ $1,200/subject = $28,800

Total Pathology Costs for Entire Period: $76,800

Animal Replacement:

AIM1:

- 24 subjects @ $6,525/subject = $156,600

AIM2:

- 24 subjects @ $6,525/subject = $156,600

AIM3:

- 24 subjects @ $6,525/subject = $156,600

Total Animal Replacement Costs for Entire Period: $469,800

Animal Per Diems:

Costs include housing, feeding, environmental enrichment and veterinary care.

Screening (AIMs 1, 2 & 3):

- 100 subjects assigned for 1 day @ $14/day = $1,400

AIM1:

- 24 subjects assigned for 180 days @ $14/day = $60,480

AIM2:

- 24 subjects assigned for 180 days @ $14/day = $60,480

AIM3:

- 24 subjects assigned for 270 days @ $14/day = $90,720

Animal pre-assignment physicals and clinical blood work (AIMs 1, 2 & 3):

- 72 subjects @ $150/subject = $10,800

Total Animal Costs for Entire Period: $222,480

Bromodeoxyuridine (BrdU):

Cost of purchasing BrdU

AIM 3

- 24 subjects @ 100 mg/kg/day for 5 days @ 4 kg = 2 gm/subject= 48 grams
- 48 grams @ $420/5 grams = $4,200

Total BrdU Costs for Entire Period: $4,200

Equipment:

The School of Medicine & Public Health at the University of Wisconsin Madison has offered support of the R01 application by committing to the purchase of two additional pieces of equipment.

Non-metallic mobile surgical lamp to perform surgeries in the magnetic resonance imaging (MRI) room:

- 1 lamp @ $3,020 = $3,020

Light source and filters for fluorescence addition to LCM microscope:

- 1 addition @ $9,480 = $9,480

Total Equipment Costs for Entire Period: $12,500

Computers:

Costs include maintenance and upgrading computer hardware, software, and peripheral items.

Total Computer Costs for Entire Period: $19,200

Data Storage:

Data storage costs include regular backup and data security. The amount of storage needed each year represents increases in the costs as the data set grows.

- Year 1: 0.25 TB @ $2,000/TB = $500
- Year 2: 0.25 TB @ $2,000/TB = $500
- Year 3: 1 TB @ $2,000/TB = $2,000
- Year 4: 1 TB @ $2,000/TB = $2,000
- Year 5: 1.5 TB @ $2,000/TB = $3,000

Total Data Storage Costs for Entire Period: $8,000

Publication costs:

Publication costs are requested for $2,000 in Year 3 and $3000 in year 5.

Total Publication Costs for Entire Period: $ 5,000

Travel:

Travel cost is requested for the PI and research staff to present findings at neuroscientific and psychiatric conferences.

Total Travel Costs for Entire Period: $6,000

Sub Contract:

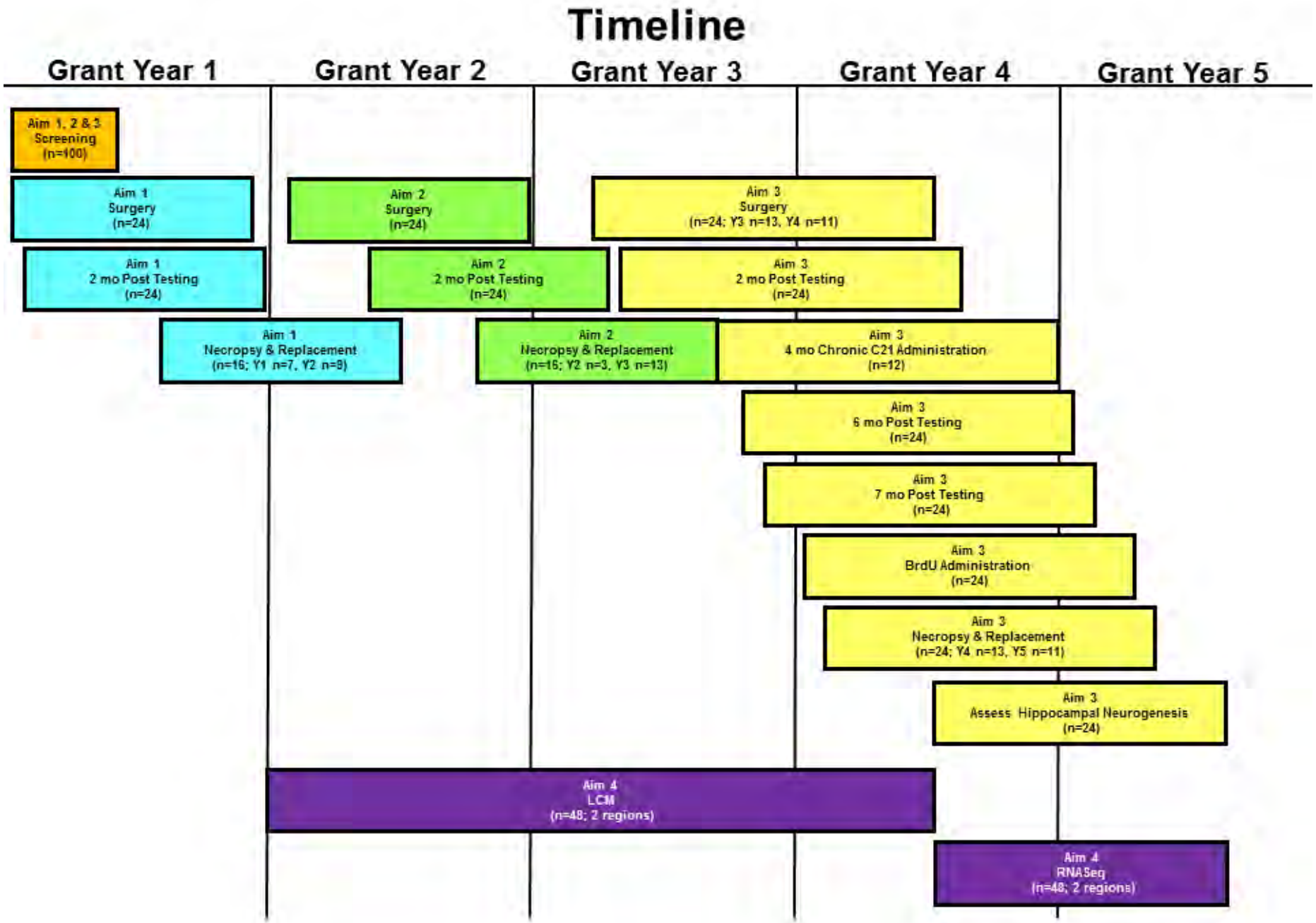
Three subawards are associated with this multisite project. Please see subaward budget justifications for specific details.

Total Sub Contract for Entire Period: $354,320

Departmental Match:

The Department of Psychiatry at the University of Wisconsin Madison has offered $25,000 per year in flexible funding to be used for grant related expenses.

Total UW-Madison Direct Costs for Entire Period: $2,278,478



RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		266,092.00
Section B, Other Personnel		295,063.00
Total Number Other Personnel	26	
Total Salary, Wages and Fringe Benefits (A+B)		561,155.00
Section C, Equipment		
Section D, Travel		4,500.00
1. Domestic	4,500.00	
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		2,067,145.00
1. Materials and Supplies	54,850.00	
2. Publication Costs	5,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	354,321.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	693,680.00	
9. Other 2	326,400.00	
10. Other 3	632,894.00	
Section G, Direct Costs (A thru F)		2,632,800.00
Section H, Indirect Costs		1,247,344.00
Section I, Total Direct and Indirect Costs (G + H)		3,880,144.00
Section J, Fee		

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

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

ORGANIZATIONAL DUNS*: 072933393

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Southern California

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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 .		name	[redacted]	[redacted]		Other (Specify)		%effort			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	name [redacted]_BJ1024225496.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 072933393

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Southern California

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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 .		name	[redacted]	[redacted]		Other (Specify)		%effort [redacted]			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
					Total Salary, Wages and Fringe Benefits (A+B)		**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	[redacted]_BJ1024225496.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 072933393

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Southern California

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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 .		name	[redacted]	[redacted]		Other (Specify)		%effort [redacted]			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	name [redacted]_BJ1024225496.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 072933393

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Southern California

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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 .		name	[redacted]	[redacted]		Other (Specify)		%effort			0.00	0.00	0.00

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	name [redacted]_BJ1024225496.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 072933393

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Southern California

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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 .		name	[redacted]	[redacted]		Consortium PI	base salary & percent effort				9,255.00	2,878.00	12,133.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **12,133.00**

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	Scientist	percent effort			8,000.00	2,488.00	10,488.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**10,488.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**22,621.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 072933393
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Southern California

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,621.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	65	70,621.00	45,904.00
		Total Indirect Costs	**45,904.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**116,525.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	[redacted]_BJ1024225496.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

USC BUDGET JUSTIFICATION

According to a DHHS rate agreement dated 06/23/2014, the USC fringe benefit rate is 31.1% and the F&A rate is 65%. Salaries are capped at the NIH Executive Level II cap where relevant.

PERSONNEL

name **M.D., Ph.D., Principal Investigator (**percent effort **in Year 5).** Dr. name/identifier He is both a board-certified psychiatrist and a well-established human geneticist with years of experience in large scale collaborations. He has cloned the genes for 2 Mendelian disorders, Retinitis Pigmentosa and Primary Pulmonary Hypertension (PPH) (Banerjee et al., 1998; Deng et al., 2000b) and continues to work on the genetics of PPH, along with the genetic basis of multiple psychiatric disorders (Nicotine Addiction, Opiate Addiction, Early-Onset Depression, Panic Disorder and Obsessive-Compulsive Disorder). His laboratory is facile with the techniques for determining the function of disease genes (transgenic mice models, cell culture, yeast two-hybrid screening, *in situ* hybridization, confocal microscopy, etc.) from the study of the Primary Pulmonary Hypertension gene (BMPR2). As described in the research plan, Dr. name will be responsible for the scientific progress and quality of the RNA sequencing portion of the project. He will also be responsible for management of the budget for this portion of the project. He will meet weekly with laboratory staff and Dr. name to oversee the work, and will have monthly conference calls with the UW site to discuss progress. Please note: actual salary is above NIH cap and available upon request.

name **PhD, Research Associate (**percent effort **in Year 5).** name will maintain the GT-FAR "pipeline" software for the analysis of Rhesus data, perform the analysis of the RNA-Seq data from the 96 samples.

SUPPLIES

Based on the need to process 96 samples, total supply costs are $48,000 ($500/sample).

RNA Sequencing Library Construction. $20,160 ($210 x 96 samples) is requested in Year 5 to purchase NuGen Ovation RNA-Seq kits and Ovation Rapid DR kits with sample indexing, both required to synthesize cDNA and build libraries, including cartridges for automated library construction on Mondrian micro fluidic instrument (NuGen).

RNA Sequencing Reagents. $27,840 ($290 x 96 samples) is requested in Year 5 for reagents to perform sequencing on an Illumina HiSeq2500 sequencer. We will perform single-end 100 bp reads using Rapid flow cells. We propose at least 40 million reads sequencing depth, taking into account that we will collect the information from both coding and non-coding RNA. Due to variation in concentration of samples in pools, minimal threshold of 40 million reads corresponds to approximately 50 million reads per sample on average.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		12,133.00
Section B, Other Personnel		10,488.00
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		22,621.00
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		48,000.00
1. Materials and Supplies	48,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		70,621.00
Section H, Indirect Costs		45,904.00
Section I, Total Direct and Indirect Costs (G + H)		116,525.00
Section J, Fee		

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

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

ORGANIZATIONAL DUNS*: 078861598

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
name					Consortium PI	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**25,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	69.5	25,000.00	17,375.00
		Total Indirect Costs	**17,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**42,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification_J[redacted]_R011024154824.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 078861598

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
name					Consortium PI	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**25,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	69.5	25,000.00	17,375.00
		Total Indirect Costs	**17,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**42,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification_[redacted]_R011024154824.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*: 078861598

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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 ($)*	Funds Requested ($)*
[redacted]					Other (Specify)	[redacted] base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	69.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification_[redacted]_R011024154824.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 078861598

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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 ($)*	Funds Requested ($)*
[redacted]					Other (Specify)	[redacted]				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	69.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification[redacted]_R011024154824.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 5

ORGANIZATIONAL DUNS*: 078861598

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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 ($)*	Funds Requested ($)*
name					Other (Specify)	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

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

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 078861598
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Mount Sinai School of Medicine

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	69.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification[redacted]R011024154824.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

BUDGET JUSTIFICATION

[redacted] Lab at Icahn School of Medicine at Mount Sinai

PERSONNEL

[redacted] (Other Significant Contributor, [redacted]. [redacted] has developed the second generation of DREADD ligands including compound 21. For this project, [redacted] will supervise the post-doctoral fellow listed below to synthesize 52.7 grams of compound 21 and 9.5 grams of CNO for the proposed in vivo studies. [redacted] will devote [redacted] effort per year for years 1 and 2, which will be cost shared. [redacted] is not requesting any salary support on this project.

Post-doctoral Researcher (to be named in [redacted] lab, [redacted] for years 1 and 2). The postdoctoral fellow will carry out large-scale syntheses to deliver 52.7 grams of compound 21 and 9.5 grams of CNO to Dr. Kalin's lab. He or she will devote [redacted] effort per year for years 1 and 2 to this project.

SUPPLIES

Reagents and lab supplies: $5,032 per year for years 1 and 2 to cover chemicals including starting materials, reagents, and solvents (anhydrous, HPLC, and regular).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		0.00
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		50,000.00
1. Materials and Supplies	50,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		50,000.00
Section H, Indirect Costs		34,750.00
Section I, Total Direct and Indirect Costs (G + H)		84,750.00
Section J, Fee		

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

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

ORGANIZATIONAL DUNS*: 041294109

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Rochester

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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 ($)*	Funds Requested ($)*
[redacted]			name	M.D.	Other (Specify)	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Budget_justification[redacted]20161024225501.pdf
(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 041294109

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Rochester

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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 ($)*	Funds Requested ($)*
name				M.D.	Other (Specify)	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification[redacted]20161024225501.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 041294109

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Rochester

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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 ($)*	Funds Requested ($)*
[redacted]				M.D.	Other (Specify)	base salary & percent effort				0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **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	

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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification[redacted]20161024225501.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 041294109

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Rochester

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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 ($)*	Funds Requested ($)*
name				M.D.	Consortium PI	base salary & percent effort				4,263.00	1,121.00	5,384.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **5,384.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
1	Graduate Students				15,527.00	0.00	15,527.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist				7,465.00	2,090.00	9,555.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**25,082.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**30,466.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

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

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**34,466.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	54	34,466.00	18,612.00
		Total Indirect Costs	**18,612.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**53,078.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name:
	Budget_justification[redacted]20161024225501.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

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

ORGANIZATIONAL DUNS*: 041294109

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Rochester

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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 ($)*	Funds Requested ($)*
name				M.D.	Consortium PI	base salary & percent effort				7,319.00	1,925.00	9,244.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **9,244.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	percent effort					
1	Graduate Students				31,986.00	0.00	31,986.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist				15,378.00	4,306.00	19,684.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**51,670.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**60,914.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **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	
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: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

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

ORGANIZATIONAL DUNS*: 041294109
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Rochester

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	4,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Per subject reimbursement	0.00
9 . Start up costs	0.00
Total Other Direct Costs	**4,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**64,914.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	54	64,914.00	35,054.00
		Total Indirect Costs	**35,054.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**99,968.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Budget_justification[redacted]0161024225501.pdf
(Only attach one file.)

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

Tracking Number: GRANT12114834
Funding Opportunity Number: PA-13-302 . Received Date: 2016-03-07T17:09:45.000-05:00

BUDGET JUSTIFICATION

Graduate student, TBD ([redacted] in Year 4, [redacted] in Year 5) will assist in processing tissue for cellular markers and/or bromodeoxyuracil immunoreactivity. He or she will also perform double and triple immunofluorescent labeling to determine the phenotype of dividing cells in hippocampus, and perform established microscopic protocols and statistical analyses related to the cell counting studies. He or she will also learn how to delineate specific subregions of the hippocampus based on cytoarchitectural and histochemical criteria, and be involved in all manuscript preparation.

[redacted] **Research Assistant Professor** ([redacted] in Year 4, and [redacted] in Year 5)) will be involved in design and monitoring of cell quantification studies, under supervision of [redacted]. With the graduate student, she will oversee all immunocytochemical staining protocols, particularly double and triple labeling to ensure that all markers are adequately characterized and studies are controlled. She will also assist with all statistical analyses and manuscript preparation.

[redacted] [redacted] in Year 4, and [redacted] in Year 5) will supervise all aspects of the post-mortem studies. She will be directly involved in mentoring the graduate student and [redacted] in designing and performing microscopic and cell counting analyses, and be available for trouble-shooting. [redacted] will also provide teaching and assistance with perfusions (performed in Wisconsin), and tissue preparation (in Rochester). [redacted] and the graduate student will meet at least every other week to review data and share progress with the team in Wisconsin.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		14,628.00
Section B, Other Personnel		76,752.00
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		91,380.00
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		8,000.00
1. Materials and Supplies	8,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		99,380.00
Section H, Indirect Costs		53,666.00
Section I, Total Direct and Indirect Costs (G + H)		153,046.00
Section J, Fee		

Total Direct Costs less Consortium F&A

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

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	499,798	499,846	499,885	499,633	499,318	**2,498,480**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:	Dr.
First Name*:	NED
Middle Name:	H
Last Name*:	KALIN
Suffix:	MD

2. Human Subjects

Clinical Trial? ❍ No ❍ Yes

Agency-Defined Phase III Clinical Trial?* ❍ No ❍ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ❍ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ❍ Yes ● No

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

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

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

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:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

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

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	DREADDs_grant_ResearchStrategy_FINAL_11024225439.pdf
3. Research Strategy*	Research_Strategy_Final1024276475.pdf
4. Progress Report Publication List	Progress_Report_Publication_List_Final1024276503.pdf
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	Vertebrate_Animals_Section_03_2_161024225547.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	Consortium_LOI1024276525.pdf
12. Letters of Support	Los_Final1024276419.pdf
13. Resource Sharing Plan(s)	RESOURCE_SHARING_PLAN_EMOTIONS_20161024225548.pdf
Appendix (if applicable)	
14. Appendix	

Specific Aims:

Anxiety disorders are major contributors to worldwide suffering, are more prevalent in females, and despite current treatments remain a major public health concern. Anxiety disorders often begin with early-life dispositional anxiety that can lead to the development of depressive disorders and co-morbid substance abuse. Treatment strategies aimed at early-life anxiety have the potential to prevent this life-long suffering. The nonhuman primate (NHP) model of early-life dispositional anxiety, or anxious temperament (AT), is ideal because of similarities between rhesus monkeys and humans in the development of socio-emotional behavior and its underlying neural circuits, which are characterized by a high degree of integration between subcortical structures and the primates' well-developed prefrontal cortex. The AT model has allowed us to uncover neural and molecular substrates that contribute to AT. Our work, and that of others, strongly implicates altered function of the extended amygdala as a core feature of AT and anxiety disorders. The extended amygdala includes the central nucleus of the amygdala (Ce), located within the dorsal portion of the amygdala, and the bed nucleus of the stria terminalis (BST), near the ventral striatum. The extended amygdala integrates threat-relevant information from cortical and subcortical inputs, and initiates behavioral and physiological responses to threat. Of particular interest are the orbitofrontal "regulatory" influences on extended amygdala function and anxiety. Despite the established role of these regions in anxiety, there are a number of critical questions in considering the development of new treatments. Advances in molecular technologies for reversibly and bi-directionally controlling brain function are beginning to make some of these questions tractable. Designer receptors exclusively activated by designer drugs (DREADDs) are ideal for examining early-life AT in NHPs because they can modulate critical brain regions for long periods of time (i.e. hours) -- particularly relevant for uncovering mechanisms related to mood and anxiety dysregulation. The DREADDs technique involves infecting a brain region with a viral vector that expresses a receptor that does not naturally occur in the brain, which is then combined with a pharmacological intervention, an otherwise "inert" drug that selectively activates DREADDs. Importantly, DREADDs can be used to chronically alter circuit function to model long-term brain alterations associated with psychopathology. We established a Cre-Lox recombination strategy to express DREADDs in NHP amygdala neurons that project to select effector sites. This allows unprecedented control of specific projections in the brains of freely behaving NHPs during exposure to ethologically relevant contexts. Because of the marked prevalence of anxiety and depression in females this proposal will study young female NHPs. We will use DREADDs to address four important questions that will provide unique evidence with the potential to guide development of new treatments for patients suffering from anxiety.

Aim 1: Understand how, early in life, projections in the extended amygdala drive sustained anxiety-related behavior and its neural substrates in young female primates. In separate groups of young female NHPs, we will selectively activate and inhibit neurons originating in the Ce region that project to BST (n=24 females, n=8 per group including shams). Our dual-infection approach uses Cre-dependent inhibitory or excitatory DREADDs, which will be infused into the Ce, while the retrogradely transported CAV2-Cre virus will be infused into the BST. Assessing effects with multimodal neuroimaging and in-depth behavioral testing will determine the causal role of Ce to BST projections on early-life AT and anxiety-related brain function.

Aim 2: Test a hypothesized role for direct projections from caudal orbitofrontal cortex (orbital proisocortex/anterior insular cortex; OPro/AI) to Ce in anxiety regulation by selectively manipulating this projection and observing its influence on anxiety-related behavior and brain function. Using the Cre-dependent DREADDs dual-infection strategy similar to Aim 1, we will selectively activate and inhibit neurons in OPro/AI that project to the Ce (n=24 females; n=8 per group including shams). Based on our metabolic imaging and lesion studies, we hypothesize that excitation of the OPro/AI to Ce projections will increase AT and extended amygdala metabolism, whereas inhibition of OPro/AI projections will have the opposite effect.

Aim 3: Explore whether chronic early-life activation of Ce is sufficient to induce extreme anxiety accompanied by the functional and structural brain changes associated with stress-related psychopathology. Twelve young females will be injected with activating DREADDs into the Ce and 12 will receive sham surgery. Six animals in each of these groups will undergo daily treatment with a designer drug for 4 months. We predict that anxiety-related changes, such as increased BST metabolism, decreased integrity of the uncinate fasciculus, and decreased hippocampal neurogenesis will be evident after chronic activation. This study will clarify the aspects of long-term anxiety that are caused by chronic Ce activation.

Aim 4: Identify molecular markers of projection-specific anxiety-modulating neurons that will enable development of selective circuit-based treatment approaches. Using post-mortem tissue from Aims 1 & 2, we will laser-capture microdissect pathway-specific (Ce to BST; OPro/AI to Ce) and adjacent non-pathway-specific neurons originating from the same region for RNA sequencing. This will allow us to compare gene expression levels between BST-projecting and non-BST-projecting Ce neurons. We will focus our analyses on cell surface receptors that could provide drugable targets. Based on our data, we predict that BST-projecting Ce neurons will overexpress the growth factor receptor gene *NTRK3*. Corresponding analyses will be performed to identify transcripts selective to the OPro/AI neurons that project to the Ce. This strategy represents a novel pathway to drug discovery and may provide a way to prevent the consequences of chronic Ce-hyperactivation.

Together these four aims will set the stage for the feasibility of amygdala projection-specific treatments for early-life anxiety, and demonstrate the utility of an animal model to test these novel approaches.

a) **Significance**

Anxiety and depression are very common disorders in adults that are associated with significant morbidity, mortality, and a high financial burden to society (*1, 2*). These disorders are also among the most prominent childhood and adolescent psychiatric illnesses and increased focus has been placed on their early onset (*3*). It is likely that most adult anxiety and depression emerges early in life but commonly goes unrecognized and untreated. Due to a lack of understanding of underlying pathophysiology, current treatments are often ineffective. Inadequate treatment of childhood symptoms results in greater functional disability, chronicity, and underlies many of the long-term consequences of psychopathology. Risk factors have been identified and provide some clues about underlying mechanisms (*4, 5*). Persistent and high levels of sustained anxiety, or anxious temperament (AT), during childhood are one of the strongest risk factors for the later development of stress-related psychopathology (*6-8*). This may be particularly relevant for high AT young females because with the transition to adolescence females are twice as likely to have anxiety and/or depressive disorders (*9-13*). Understanding the mechanisms that underlie early-life dispositional anxiety in females, how these neural mechanisms can be altered to decrease anxiety, and understanding the mechanisms underlying the long-term consequences of brain alterations are the focus of this proposal because these are critical steps for generating novel, developmentally-appropriate treatment strategies. Because key risk factors and mechanisms cannot be prospectively manipulated in humans, causal evidence must be derived from valid animal models. Nonhuman primate (NHP) models provide a powerful means of doing so and, more generally, for bridging the translational gap between mechanistic rodent studies and clinical applications (*14*). The proposed primate mechanistic studies are aimed at manipulating inputs and outputs of a core component of the neural circuitry mediating anxiety to understand, at a microcircuit level, the distributed brain alterations characteristic of children with extreme anxiety. *Primate Anxious Temperament (AT) to Study Childhood Risk*: Our work demonstrates a high level of convergence between AT in young monkeys, AT in children, and childhood anxiety disorders. Importantly, rhesus monkeys and humans share a well-developed prefrontal cortex (PFC) (*15, 16*), which is thought to be critical for optimal regulation of anxiety (*17-21*). The AT phenotype is assessed during the mildly threatening no eye contact (NEC) condition of the human intruder paradigm (*22*). The monkey AT phenotype models early life risk for psychopathology and extends the unidimensional measure of behavioral inhibition (BI) because it combines stress-related pituitary-adrenal activity (cortisol) with multiple behavioral indices, i.e. freezing and vocalizations. AT reflects a broad context-independent anxious disposition that is stable over time (*23-25*), predicts brain metabolism across contexts (*24*), as well as other anxiety-like behaviors that span multiple contexts, including “calls for help” during separation (r=-0.52, p<.001), barking in response to direct eye-contact (r=-0.25,p<.005), and the latency to reach for a food reward during exposure to a novel but neutral object (ρ=.43, p=.01). These data provide compelling evidence that our composite AT construct is a context-independent, multi-dimensional phenotype similar to that observed in children with anxiety disorders, and those with extreme AT who are at marked risk for the development of stress-related psychopathology. As in children (*26-30*), monkey AT is identifiable early in life, stable across development, and heritable (*23, 24, 31*). Data from our lab and others also show that monkey AT and childhood anxiety share similar patterns of pituitary-adrenal activity (*24, 31-34*) and frontal EEG asymmetry (*35-44*). Additionally, the administration of anxiolytic GABAergic drugs decrease AT while normalizing EEG asymmetry (*22, 36, 37*). Collectively, these data underscore the validity of the monkey AT model and substantiate its relevance to the human childhood risk to develop anxiety and depressive disorders.

Neural Circuitry Underlying AT: Our work, and others, has highlighted a distributed neural system thought to underlie adaptive and maladaptive anxiety that include anterior hippocampus, orbitofrontal cortex (OFC), anterior insula (AI), and the extended amygdala (*23, 31, 45-47*) (Figure 1). The central nucleus of the amygdala (Ce) and the bed nucleus of the stria terminalis (BST) are core components of the extended amygdala (*48-52*). The Ce is a sub-nucleus of the amygdala located in the dorsal amygdala. Our selective lesion study demonstrated that the Ce is a critical causal substrate

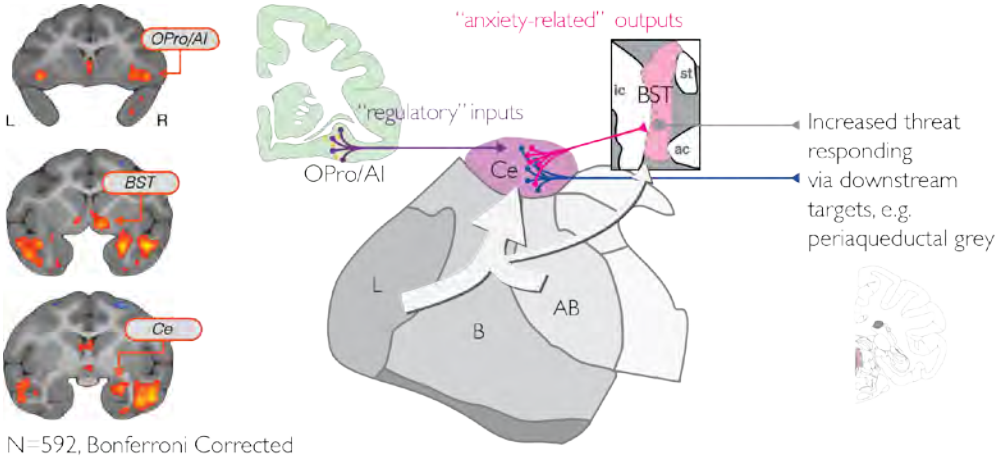


Figure 1. FDG-PET imaging in 592 young NHPs reveals OPro/AI, BST and Ce as components of AT’s neural substrates (left, 23). In our working model we focus on OPro/AI’s direct “regulatory” input to Ce (dark purple), and Ce’s threat-related outputs, including “anxiety-related” outputs to BST (dark pink). Ce and BST are part of the extended amygdala that modulates threat-responding via downstream targets in brainstem and hypothalamus.

of AT (*53*). The BST shares strong functional and structural connections with Ce (*54, 55*), is composed of Ce-like cell types (*49, 50, 56*), and is located near the ventral striatum around the anterior commissure. The Ce and BST share efferent targets, including the brainstem and hypothalamus, that are critical to mounting fear and anxiety responses (*57-60*), and integrate emotional information from cortical and subcortical regions to initiate and maintain threat-related responding (*45*). Interestingly, in our work with name we demonstrated that primate BST and Ce are asymmetrically connected, such that most connections emanate from Ce to BST (*61*). Thus, we, and others, hypothesize that coordinated function between Ce and BST, likely resulting from Ce projections to BST, play an important role in mediating adaptive and maladaptive anxiety responses. Human imaging studies examining anxious traits and anxiety disorders implicate numerous brain regions, including the extended amygdala and OFC (*62-64*). Similarly, using high-resolution ^{18}fluoro-deoxyglucose PET (FDG-PET) scanning in 592 young NHPs from a multigenerational pedigree, we demonstrated the importance of the primate extended amygdala and OFC in predicting AT (*23, 31*) (Figure 1). These findings are particularly relevant, as the extended amygdala receives afferent projections from the caudal OFC and anterior insular regions (*65-69*). Consistent with the stability and trait-like features of AT, we showed that individual differences in metabolism in key nodes of the AT neural circuit were stable over time and across contexts (*23, 24, 70*). In addition, we found evidence for differential heritability of the components of AT's neural substrate, and a significant genetic correlation between AT and metabolism within extended amygdala and specific caudal OFC regions encompassing portions of orbital proisocortex and anterior insula (OPro/AI) (*23, 31*).

Our monkey lesion studies revealed that selective destruction of OFC decreases AT (*46, 71*). Price (*72-74*) has characterized the OFC as comprised of orbital and medial networks. Whereas both medial and orbital networks are involved with emotion processing, our NHP studies targeted caudal orbital areas, including OPro/AI, because of their dense bidirectional connections with the amygdala (*72-74*). This includes direct projections to extended amygdala, and their putative involvement in emotion regulation (*17-21*). The OPro/AI region of the caudal OFC is particularly interesting because it directly projects to Ce (*75-78*) and in our genetic correlation analyses was implicated in the intergenerational transmission of anxiety (*23*). Using FDG-PET to study the downstream effects of OFC lesions, we found evidence consistent with a regulatory role for the monkey OFC such that these lesions led to chronically attenuated metabolic activity in the extended amygdala (*71*). Moreover, variation in activity in the extended amygdala predicted individual differences in lesion-induced reductions in AT. Our findings extend early insights gleaned from the study of Phineas Gage (*79, 80*) and complement the results of more recent human lesion (*81-87*) and imaging studies (*62, 63, 88, 89*). For instance, similar to our NHP studies, patients lacking portions of their OFC had decreased extended amygdala blood flow (*90*). Furthermore, recent fMRI studies in adults with generalized anxiety disorder suggest altered functional and structural connectivity between amygdala and OFC (*91, 92*). Together, these data provide a compelling rationale for exploring the function of specific "anxiety-related" Ce to BST and "regulatory" OPro/AI to Ce projections in the genesis of early-life anxiety.

Molecular Substrates of AT: NHP RNA-Seq: Our extensive collaboration with the name laboratory positions us to identify molecular alterations within the specific circuits that we hypothesize are critically involved in mediating AT (i.e., Ce to BST and OPro/AI to Ce projections). Through this collaboration, we have engaged in systematic gene expression studies focused on understanding altered gene expression in the Ce, the core neural component of AT. Initially we began with microarray studies of punch biopsies (*93, 94*) and more recently have used next generation deep RNA sequencing (RNA-Seq) of laser capture microdissected (LCM) neurons harvested from Ce. Our initial studies implicate predicted genes (e.g. *NPY1R*, *HTR2C*, *GABRA5*) as well as unpredicted neuroplasticity-related molecular alterations that were associated with AT and increased Ce metabolism. We found that reductions in the expression of genes related to the NTF3 (neurotrophin-3)-NTRK3 (neurotrophic tyrosine kinase receptor-3, also termed TrkC) pathway predicted increased levels of anxiety. NTRK3 is of particular interest because it is a cell surface receptor that can initiate synaptogenesis and neurogenesis (*95*). These findings formed the basis of a new hypothesis suggesting that reduced Ce neuroplasticity during development underlies the later risk to develop anxiety and depression (*93, 96*). These findings add to recent reports demonstrating that in addition to the hippocampus, neuroplasticity mechanisms in the Ce are functionally relevant to anxiety-related responses (*97, 98*). We are currently analyzing data sets from Ce neurons harvested with LCM. The current proposal (Aim 4) will extend these studies by identifying transcripts that are specifically expressed in critical subsets of neurons within Ce and OPro/AI that will be identified from the NHPs in Aims 1 & 2. Together, these data will allow us to identify highly-specific AT-related transcripts in Ce and OPro/AI that will serve to guide future mechanistic molecular studies, aimed at developing novel drug targets that will selectively manipulate critical AT-related neural circuits.

Progress Report: Mechanistic Molecular Studies in NHPs: During the previous funding period (7/2012 – present), in our NHP model of AT we have developed the capacity to combine a constellation of state-of-the-art techniques, including functional imaging, viral vector transduction methods, MRI-guided surgery, sophisticated behavioral analyses, and post-mortem molecular analyses including assessment of neurogenesis, to characterize the mechanisms relevant to early-life anxiety. We have developed reliable techniques to effectively and efficiently infect neurons in targeted, small brain regions with viral vectors using intraoperative MRI combined with gadolinium (Gd) enhanced visualization (Figure 2). We have performed over 30 surgeries

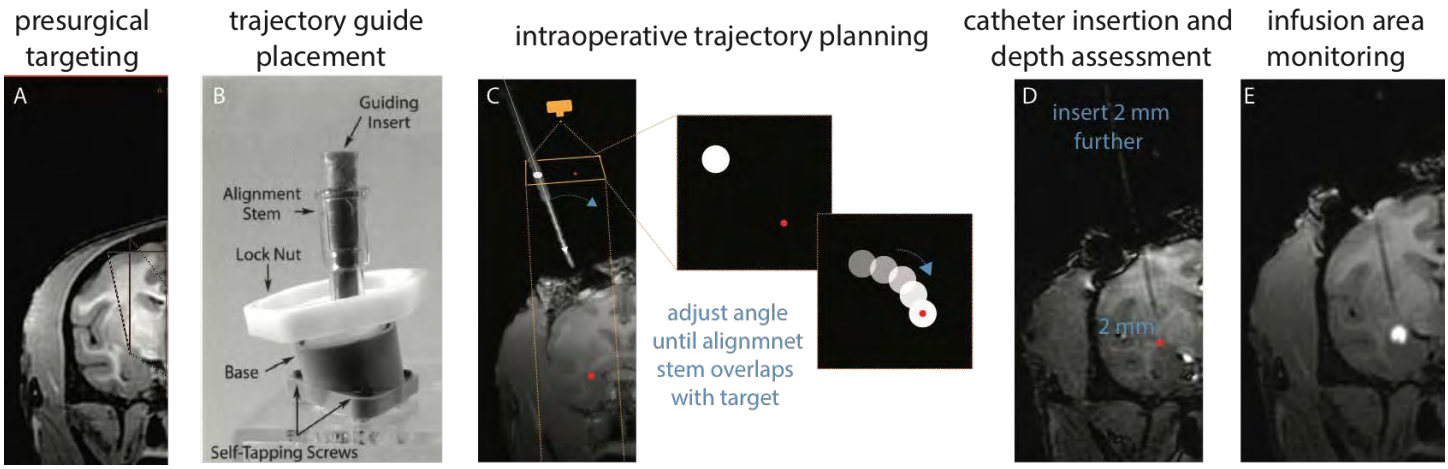


Figure 2. Real-time intraoperative MRI guided targeting and infusion monitoring. Prior to surgery a structural MRI is obtained to visualize the target and plan the trajectory (*a*). A pivot point-based MRI compatible external trajectory guide is mounted on the skull (*b*). Precise targeting is performed by imaging a plane orthogonal to the long axis of the external trajectory guide, as if it were visualized by a camera from above (*c*). The inset boxes represent the plane in which the trajectory guide (white dot) is visualized and aligned in relation to the target injection point (red dot). The depth of the catheter is advanced to approximately 2 mm above the target. Another MRI is acquired to make precise measurements between the catheter tip and the target prior to advancing the catheter to its final position (*d*). Infusion of viral vector infusate with gadolinium (Gd) is monitored in real time, in this case verifying the infusion into the targeted Ce region (*e*).

during the past 2.5 years using convection enhanced delivery (CED) and intraoperative MRI to target AT-related circuitry, including Ce, BST, and OFC regions. In accomplishing the aims of the previous funding period, we have used viral-vector strategies to produce long-term alterations (i.e. >1 year) in the expression of genes having a putative role in mediating AT, including those we identified through our RNA sequencing studies. By overexpressing the stress-related neuropeptide corticotropin-releasing hormone (CRH) in the NHP Ce, we demonstrated increases in AT, decreases in hippocampal neurogenesis, and, with neuroimaging, found structural and functional alterations throughout AT's neural circuit (*99*). In contrast, by overexpressing a neuroplasticity-related gene, Neurotrophin-3 (NT3), in the Ce we found a decrease in NHP AT and changes in the function of its neural circuit (unpublished data). While informative, these overexpression studies are limited by their chronic and irreversible nature.

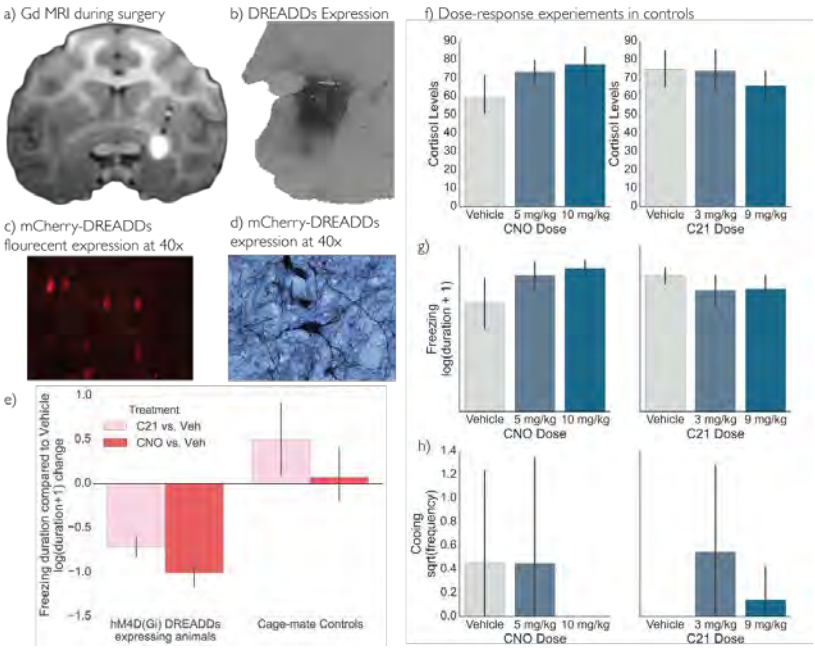


Figure 3. Image of dorsal amygdala AAV5-hM4D(Gi)-mCherry DREADDs vector mixed with Gadolinium (Gd) as observed with intraoperative MRI (a), and post-mortem validation using mCherry antibody (b & d) and fluorescence (c). DREADDs mediated reductions in freezing relative to vehicle after CNO (red) and C21 (pink) compared to controls (n=3/group; e). Lack of significant effects on AT (Cortisol, f; Freezing, g; Cooing, h) in control animals (n=5/group) treated with CNO (left) or C21 (right).

DREADDs a New Frontier: NHP Studies: Advances in molecular biology over the last decade have provided tools for the reversible manipulation of brain circuits allowing for unprecedented insights into circuit-based mechanisms underlying complex behaviors. Deisseroth, Boyden, and colleagues have pioneered optogenetic tools, to express channel rhodopsins in neurons that can be activated or inhibited by different wavelengths of light (*100-103*). In parallel, Roth and colleagues have developed a set of chemogenetic tools, to express designer receptors exclusively activated by designer drugs (DREADDs) that also allow for reversible manipulation of neural circuits (*104, 105*). The initial DREADDs, which are featured in this proposal, were derived from human M3 and M4 muscarinic receptors that were modified to retain G-coupled inhibitory/excitatory intracellular signaling while rendering the receptor unresponsive to acetylcholine. The DREADDs technique offers a number of advantages for studying AT in NHPs, which requires behavioral observations in freely moving animals over a prolonged period of time in a naturalistic setting. For testing behavioral effects, DREADDs do not require a chronic invasive preparation, such as an indwelling light source or skull removal for

light source access. Additionally, the time course of DREADDs is well suited for the prolonged 30-minute observation period used in our monkey model of AT to assess sustained anxiety and its regulation.

Researchers at NIH and UNC have taken a lead role in pioneering DREADDs techniques for reversible control of brain circuits in NHPs. A landmark paper from the NIH group recently demonstrated, for the first time, that by using viral vector strategies to express DREADDs in NHP OFC, researchers could repeatedly and reversibly alter behavior by administering the designer drug, clozapine-N-oxide (CNO), which has no physiologically-relevant binding affinity (K_i > 1 μM) for any endogenous CNS receptor (*106*). Building on our extensive RT-IMRI and viral vector expertise, in collaboration with [redacted] and [redacted], we established DREADDs methods for manipulating the amygdala of young NHPs. We have transfected numerous animals with DREADDs, and performed pilot behavioral experiments to assess the feasibility of using this technology to alter anxiety-related behavior (Figure 3). We have also performed dose-response studies with designer ligands, i.e. CNO, and Compound 21 (C21)(*107*), in control animals to assess possible off-target effects (Figure 3f-h). Although C21 has the potential for minimal off-target effects, it is of particular value when administered chronically in primate studies because, unlike CNO, there is no risk of back-metabolism into other psychoactive compounds. Our effort has benefited from our collaboration with [redacted], who is responsible for synthesizing and supplying us with CNO and C21. Initial studies using hM4D(Gi) DREADDs into the Ce-region suggest that CNO (5 mg/kg) and C21 (9 mg/kg) effectively decrease NEC-induced freezing, whereas these compounds were without significant effects in the control NHPs (Figure 3e). Dr. Kalin presented these initial data at a recent Cold Spring Harbor Laboratory meeting (October, 2015) exploring the potential for using DREADDs technology in NHPs as a translational platform for developing chemogenetic methods for clinical interventions in humans.

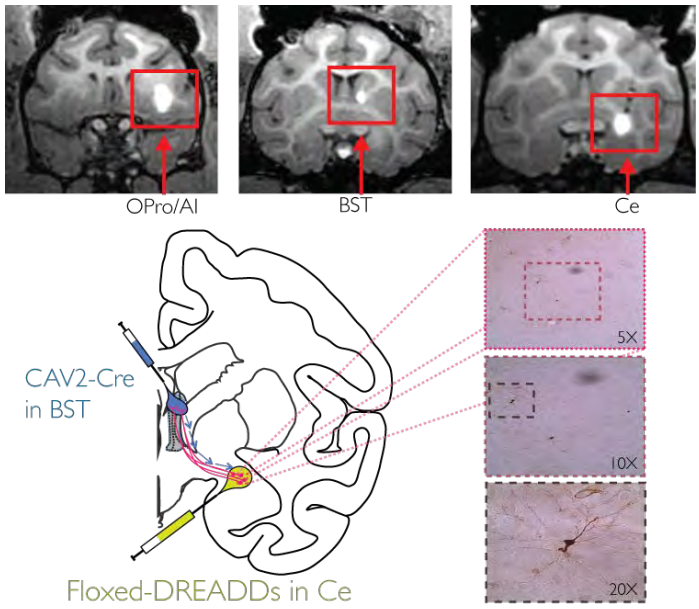


Figure 4. Injections of viral-vector mixed with Gd into OPro/AI, BST, and Ce (top) demonstrate selective targeting of these regions for our dual infection approach. For example, CAV2-Cre into BST and floxed-DREADDs into Ce resulted in projection specific DREADDs expression in Ce neurons that project to BST.

By allowing for the reversible control of specific circuits and also the ability to examine the long-term effects resulting from chronic activation of these circuits, these tools allow us to answer mechanistic questions about the neural circuits of primate anxiety that were previously untestable. We have developed methods in NHPs combining DREADDs with Cre-lox techniques to selectively express DREADDs in neurons that project from one region to another (Figure 4). We will use this dual-infection strategy to specifically activate/inhibit neurons that project from Ce to BST (Aim 1) and to specifically activate/inhibit neurons that project from OPro/AI to Ce (Aim 2). The Cre-lox technique works by infecting neurons with an adenovirus expressing floxed-DREADDs and, in a projection site of these neurons, infecting axons with the retrograde transported canine adenovirus (CAV2, (*108*)) expressing Cre recombinase (CAV2-Cre). This dual-infection technique (*109*) results in the expression of functional DREADDs in neurons within the selected region (e.g. Ce) that project to the CAV2-Cre infected projection site (e.g. BST). See Figure 4, which demonstrates our ability to successfully perform the dual infection surgeries and specifically induce pathway-specific DREADDs expression.

Approaches Taken to Ensure Robust and Unbiased Results: To ensure that our results are robust, all experiments are rigorously designed to include an age-, sex-, and cage-matched control group. To ensure that our results are unbiased, raters of anxiety behavior as well as research staff counting cells and performing hormonal assays are always blind to treatment condition. In addition, dependent variables such as freezing and cooing responses are well defined in advance and research personnel rating behaviors are tested for reliability. All animal care, behavioral testing, multimodal imaging, microdissection and cell counting are performed in highly standardized and controlled environments with strict standard operating procedure guidelines. Analytic strategies are agreed upon *a priori* and correct for multiple comparisons when appropriate.

b) **Innovation**: The extended amygdala, including Ce and BST, is a highly relevant substrate for anxiety disorders and other stress-related psychopathology. The questions we aim to address in this proposal represent issues that have eluded researchers aimed at understanding extended amygdala circuits in the primate brain. Our focus on young female NHPs is innovative, as anxious young girls are at a particularly increased risk for developing anxiety and depressive disorders, and most prior animal modeling studies have focused on males. The questions in this proposal have not been addressable using previously available

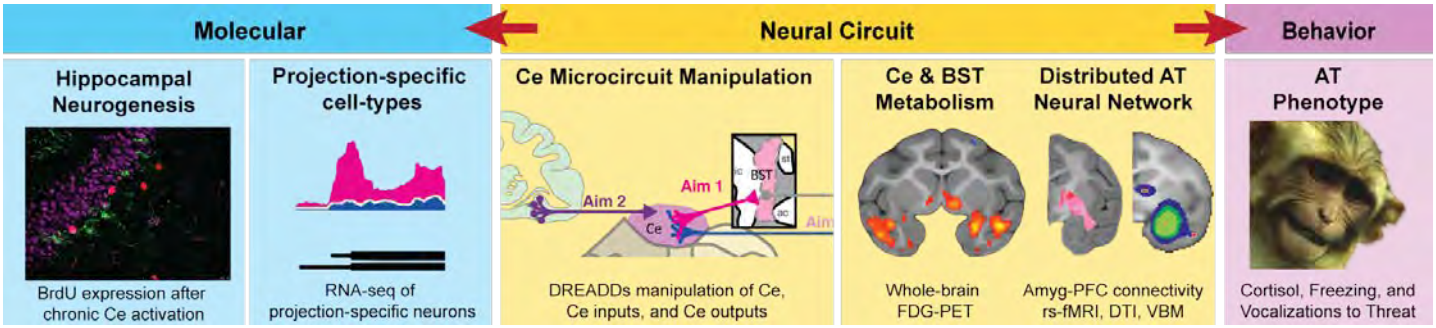


Figure 5. Our innovative and integrative approach for identifying the mechanisms and molecules underlying increased expression of AT, and the risk to develop anxiety and depressive disorders.

techniques. This proposal is poised to provide critical insights into the nature of extreme dispositional anxiety, the risk to develop anxiety and depressive disorders, and to bring the field closer to new treatments focused on modulating extended amygdala circuitry. **1**) Our chemogenetic dual-infection DREADDs approach for reversible manipulation of specific extended amygdala circuits represents the first functional dissection of anxiety-related microcircuits in the primate brain. **2**) We use a highly novel tactic to explore prefrontal control of amygdala responding. While many researchers are focused on understanding how prefrontal regions influence basolateral intra-amygdaloid circuits, little is known about the functional significance of direct OPro/AI to Ce projections. Elucidating the function of these understudied neurons may provide new insights into prefrontal contributions to extreme temperamental anxiety and anxiety disorders. **3**) Our NHP model of AT allows us to combine tools used in human neuroimaging, with reversible control of specific circuits using molecular techniques common to rodent studies. By combining these techniques, we will gain unique insights into the underlying mechanisms accounting for the functional alterations observed in adult human neuroimaging studies. By selectively activating/inhibiting neurons that project from one region to another we will elucidate how specific projections influence the function of the target region (FDG-PET), as well as their functional connectivity (rsfMRI). **4**) By chronically activating the Ce, we will, for the first time, determine the extent to which the distributed functional and structural alterations associated with stress-related psychopathology and decreased hippocampal neurogenesis can be attributed to overactivation within a specific neural circuit. **5**) Developing chemogenetic tools in the NHP is an important step toward developing targeted treatments in the human brain, which many believe is an important priority. Our focus on advancing the use of chemogenetic tools for selective and long-term control of target neurons in primates is a highly significant endeavor with direct translational potential. **6**) Identifying molecular targets in the primate that are specific to neurons in key neural circuits could provide clues for the development of new anxiolytic and antidepressant pharmaceuticals. Our unique approach using laser capture microdissection (LCM) of projection-specific neurons with RNA-Seq, combined with cutting-edge bioinformatics tools and multimodal imaging affords an unprecedented opportunity to understand the molecular composition of neurons within specific AT-related pathways. **7**) The availability of the DREADDs expressing NHPs will allow for the exploration of new methods and techniques that will further enable potential translation to humans. To this end, the UW Department of Psychiatry will commit an additional $25,000 a year to support these exploratory initiatives (see letter from UW-Psychiatry Dept). Collaborating with name we will explore tools to quantify DREADDs expression *in vivo*, such as [^{11}C]-clozapine and other radioligands for PET imaging, as well as magnetic resonance spectroscopy (MRS) to quantify GABA, glutamate, and IP3 activation. Moreover, to examine the precise electrophysiological features of the relevant Ce and OPro/AI neurons, in collaboration with name and name, we will explore the use of *in vivo* and *ex vivo* electrophysiological techniques. Together, these aims provide a programmatic and translational framework for linking function of specific neural circuit projections to dispositional anxiety, and using this information to identify novel molecular targets that can help guide the development of new treatments for adults and children suffering from stress-related psychopathology. The assembled research team is uniquely suited to accomplish the aims of this grant, as we have expertise in NHP anxiety (Kalin, name NHP brain imaging name & Kalin), MRI measures of functional connectivity (name r), DREADDs technology, electrophysiology and biochemistry (name), MRI-guided gene delivery methods (Kalin, name, LCM of primate tissue name Kalin), NHP immunocytochemistry (name and RNA sequencing and data analyses (name & Kalin).

c) **Approach**: General Experimental Design: To understand the influences Ce circuits on anxiety and its associated neural substrates young preadolescent female monkeys will be used (age ~1.5 years). We have selected young females because, in addition to extreme dispositional anxiety, being female confers additional risk for the development of stress-related psychopathology. Furthermore, we have chosen females because they are generally understudied relative to males – especially in studies using animal models. Unfortunately,

budget limitations prevent us from also studying males in this proposal. For each Aim, we will screen monkeys with the human intruder paradigm (HIP) and select those that are in the mid-range of AT. This will assure that the DREADDs experiments use a homogeneous cohort to test our hypotheses about increasing and decreasing AT. Animals will also be screened to ensure that they do not have relevant, infection-interfering, antibody titers for the AAV and CAV viruses that will be used in the experiments. CNO will be used as the pharmacological agent to acutely activate/inhibit DREADDs-expressing neurons. It is important to note, that even though CNO has, in primates, been reported to be back metabolized to its parent compound, clozapine, this will not interfere with the DREADDs experiments. While we have already established the doses of CNO and C21 that are effective and are without non-specific effects on average (Figure 3f-h), similar to Eldridge et al., (2016), we will ensure that the animals entered into the study are not affected by acute CNO administration by screening animals with CNO and selecting those that are unaffected. Selected animals will undergo RT-IMRI-guided surgery for viral vector infusions. Two months after surgery, which is ample time for the functional DREADDs receptors to be expressed, animals will begin behavioral testing and neuroimaging. Each acute test will be performed with CNO and vehicle on separate occasions, using a counterbalanced crossover design to examine DREADDs-mediated effects. There will be ~3-weeks between each acute test to ensure enough time for drug washout, and to minimize habituation. Importantly, because the time course for CNO back-metabolism, testing the acute (~30-min) effects of DREADDs activation and the use of sham-operated controls, will obviate any remaining concerns regarding non-specific effects associated with back-metabolism. The primary outcome measures will be anxiety-related behavioral, physiological, and neuroimaging measures assessed in response to a 30-minute exposure to the potentially threatening NEC condition of the HIP. All measures will be collected between 09:00-11:00 hrs to control for time-of-day. FDG-PET will be used to assess regional brain metabolism during the NEC condition (*24, 31, 47*). The primary measure, AT is calculated as the combination of NEC-induced freezing (log transformed), reductions in vocalizations (square root transformed) and plasma cortisol concentrations (residualized for time of day) (*23, 24, 31*). MRI will be used to collect structural (T1-weighted) images, diffusion weighted images (DWI), and functional connectivity with fMRI (*31, 53, 110*). We will also examine the effects of DREADDs activation/inhibition on other anxiety-related measures outlined in the methods section below. After the last assessment, animals will be sacrificed and tissue will be collected for analysis of DREADDs expression, as well as hippocampal neurogenesis after BrdU administration (Aim 3) and RNA-Seq analyses as outlined in Aim 4 (brains from animals in Aims 1 & 2).

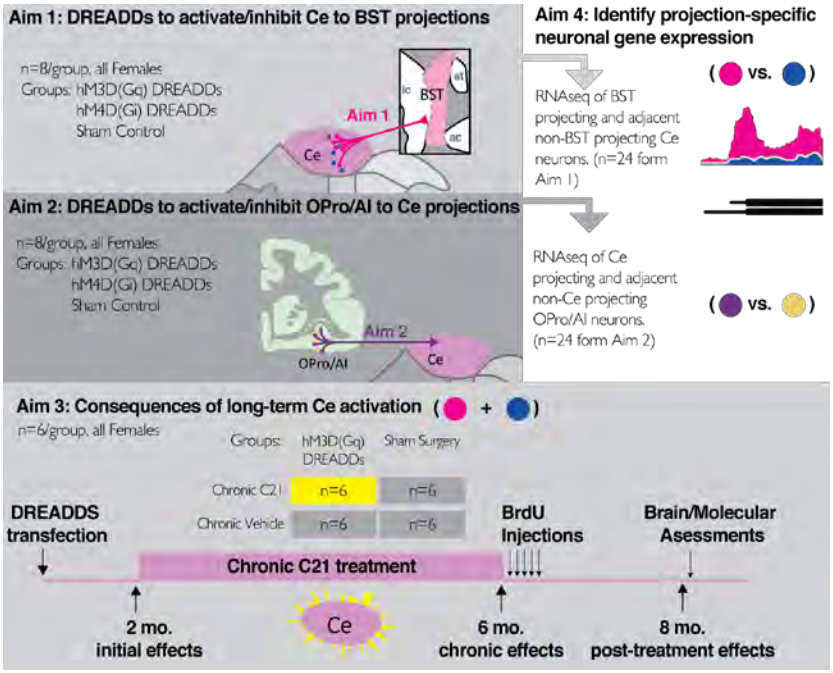


Figure 6. Experimental strategies and timeline.

Aim 1: *Understand how, early in life, projections in the extended amygdala drive sustained anxiety-related behavior and its neural substrates in young female primates.* In humans and NHPs, Ce and BST are strongly functionally connected as measured with resting-state fMRI (rsfMRI). This connectivity is predictive of AT, and the coordinated function of Ce and BST are hypothesized to be critical in integrating responses to threat (Figure 7). In NHPs the projections from Ce to BST are considerably more prominent than the reverse. Therefore, we predict that manipulating neurons in Ce that project to BST will result in alterations in AT, Ce-BST rsfMRI functional connectivity, and AT-related BST metabolism. To accomplish this Aim, three groups will undergo RT-IMRI-guided surgery: inhibitory DREADDs, excitatory DREADDs, and sham-operated controls (n=8/group). In our DREADDs groups, we will use the dual-infection Cre-lox recombination strategy, as performed in our laboratory (see Figure 4), by injecting either inhibitory AAV5-hSyn-DIO-hM4D(Gi)-mCherry or excitatory AAV5-hSyn-DIO-hM3D(Gq)-mCherry into the Ce, and, during the same procedure, injecting CAV2-Cre into the BST region. This approach will ensure the selective expression of DREADDs in Ce neurons that project to BST. Sham animals will receive CAV2-Cre into the BST region, which will be injected in combination with a Cre-recombinase-dependent fluorescent reporter into Ce in place of the DREADDs-containing virus. Animals will begin testing 2 months after surgery as outlined in the General Experimental Design section above. We predict that exciting the Ce neurons that project to the BST region will result in increased expression of AT, increased BST-metabolism, and increased functional connectivity between Ce and the BST region. In contrast, we predict that inhibiting Ce neurons that project to the BST region will have the opposite

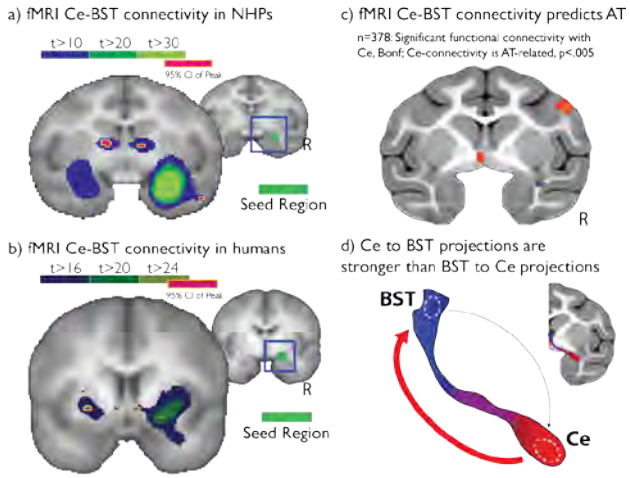


Figure 7. Monkeys (a) and humans (b) show robust Ce-BST functional connectivity as measured with resting fMRI (55, 61, 130). In 378 young monkeys, this connectivity is associated with AT (c). Our collaboration with Dr. Fudge demonstrates that most of the Ce-BST connections run from Ce to BST (d).

effect, including decreasing AT expression. This Aim provides a unique opportunity to understand the role of this projection in mediating other functional brain alterations that are commonly observed in patients with anxiety disorders. For example, we will investigate the extent to which OFC, anterior hippocampus (aHIP), and periaqueductal gray (PAG) metabolism are affected by excitation and inhibition of Ce to BST projections. At the end of the experiment animals will be sacrificed and fresh-frozen brain tissue will be collected for verification of the extent of viral vector expression using fluorescence and for LCM harvesting of neurons for RNA-Seq analyses described in Aim 4.

Aim 2: *Test a hypothesized role for direct projections from caudal orbitofrontal cortex (orbital proisocortex/anterior insular cortex; OPro/AI) to Ce in anxiety regulation by selectively manipulating this projection and observ ing its influence on anxiety-related behavior and brain function.* Prefrontal cortex is thought to play a critical role in the regulation of emotions, which is consistent with our demonstration of increased AT-related metabolism in the OPro/AI region (Figure 1). Moreover, lesions to OFC, including OPro/AI, as well as more selective strip-lesions in the OPro/AI region decrease AT (Figure 8a-d). Many OFC regions are bidirectionally connected to the amygdala, especially its ventral sub-nuclei (*77, 78, 111, 112*). Interestingly, the OPro/AI region also directly projects to Ce (Figure 9), and does not receive reciprocal innervation (*78, 111*). Though it has been largely unexplored, we propose that manipulations of this projection will have marked effects on Ce function as well as AT. To accomplish this aim, three groups will undergo RT-IMRI-guided surgery: inhibitory DREADDs, excitatory DREADDs, and sham-operated controls (n=8/group). In the DREADDs groups, we will inject OPro/AI with either inhibitory AAV5-hSyn-DIO-hM4D(Gi)-mCherry or excitatory AAV5-hSyn-DIO-hM3D(Gq)-mCherry, and, during the same procedure, inject CAV2-Cre into the Ce region. This approach will result in the selective expression of DREADDs in OPro/AI neurons that project to Ce. Sham animals will receive CAV2-Cre into the Ce region, which will be injected in combination with a Cre-recombinase-dependent fluorescent reporter into OPro/AI in place of the DREADDs-containing virus. Although many researchers have hypothesized a role for prefrontal downregulation of amygdala function, our data, and that of others suggests that OPro/AI region to be involved in the upregulation of anxiety-related responding. Moreover, our focus on the OPro/AI to Ce projection (Figure 9) is unique as opposed to the well-studied OFC to ventral amygdala projections that have been implicated in reward and fear learning (*113-116*). Animals will be tested 2 months after surgery as outlined in the General Experimental Design section above. We will test the hypothesis that inhibition of the OPro/AI to Ce projections will decrease AT. Similar to Aim 1, we will directly investigate the effects of inhibiting and exciting this projection on Ce function, as well as distributed AT-related brain function. We hypothesize that increased Ce and BST regional metabolism will result from exciting OPro/AI to Ce projections. As in Aim 1, fresh-frozen brains will be collected from animals for verification of the extent of viral vector expression and for harvesting of neurons for RNA-Seq analyses described in Aim 4.

Aim 3: *Explore whether chronic early-life activation of Ce is sufficient to induce extreme anxiety accompanied by the functional and structural brain changes associated with stress-related psychopathology.* Chronic stress commonly precipitates anxiety and depressive disorders, and is associated with a set of distributed brain alterations that include Ce hyperactivation. While researchers have suggested that increased activation of Ce may underlie many of the distributed functional and structural anxiety-related alterations, this has not been directly tested. Studies in rodents by our collaborators (Drs. [redacted] have demonstrated the utility of chronic DREADDs experiments. For example, in one study in which excitatory hM3D(Gq) DREADDs were expressed in dorsal raphe neurons, they found that chronic activation of these

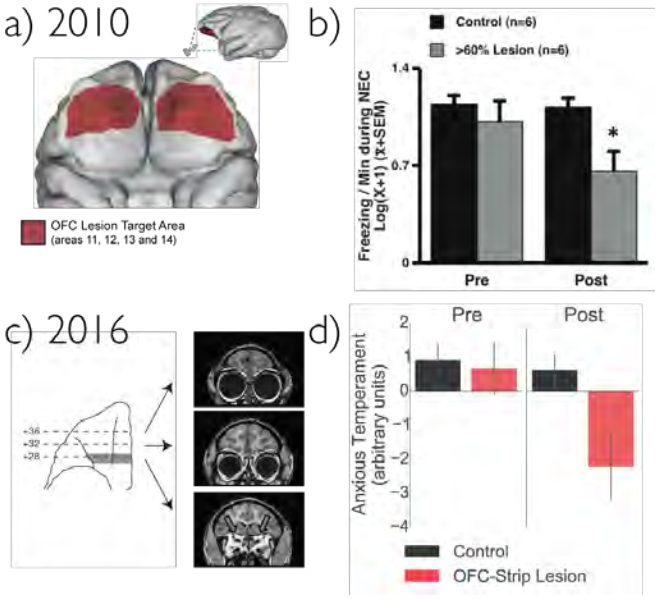


Figure 8. OFC lesions (a) result in decreased freezing (b). OPro/AI strip-lesions (c) result in decreased AT, a measure that includes freezing behavior (d).

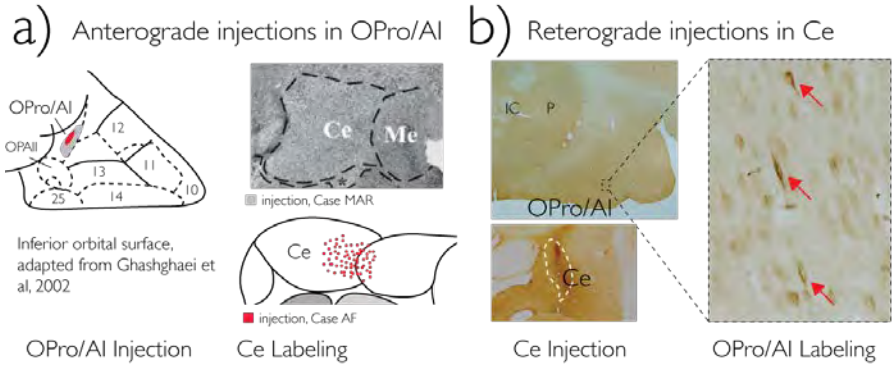


Figure 9. Anterograde (a) and retrograde (b) tract tracing demonstrates the existence of direct OPro/AI to Ce projections (75; and [redacted]).

neurons with daily CNO administration resulted in antidepressant-like effects (*117*). Furthermore, *in vitro* slice electrophysiological studies demonstrated that the chronically activated neurons remained CNO-responsive (*117*). In this proposal we aim to examine the chronic effects of Ce activation with excitatory DREADDs. To accomplish this aim, two groups will undergo RT-IMRI-guided surgery: excitatory DREADDs and sham-operated controls (n=12/group). The DREADDs group, will be injected with excitatory AAV5-hSyn-hM3D(Gq)-mCherry, into the Ce region; the sham group will be injected with AAV5 containing a fluorescent reporter. Two-months after surgery, we wil l begin treatment to chronically activate Ce. Because of the issues related to CNO back-metabolism in primates that over time could result in the accumulation of behaviorally relevant clozapine levels, Aim 3 will employ daily administration of C21. Although C21 is known to have some off-target effects (*107*), such as low-levels of binding to H_1 histamine receptors, it remains the best choice for chronic administration because it has substantially less non-DREADDs mediated effects than other DREADDs-activ ating ligands, and unlike CNO is not back metabolized into clozapine. To control for any potential effects of chronic C21 administration that are not DREADDs mediated, Aim 3 includes a sham-surgery control group that is chronically treated with C21. So that the data from Aim 3 can be directly compared with the acute experiments in Aims 1 & 2, CNO will be used as the designer drug for behavioral/neuroimaging tests. The chronic experiment will employ a 2x2 design ([DREADDs vs. sham] X [chronic C21 vs. vehicle]) for daily administration of C21 or vehicle over four months. Here, half of the DREADDs group will receive chronic C21 (hM3D(Gq)/chronic C21, n=6) and half will receive chronic vehicle (hM3D(Gq)/chronic vehicle, n=6). Similarly, half of the sham animals will receive chronic C21 (sham/chronic C21, n=6) and half will receive chronic vehicle (sham/chronic vehicle, n=6; see Figure 6 for experimental time-line). In addition, similar to our viral-vector study overexpressing CRH (*99*), after 4 months of C21 administration animals will receive bromodeoxyuridine (BrdU) daily (100 mg/kg/day) over 5 days for the later assessment of effects on hippocampal neurogenesis. Two months after BrdU administration animals will be sacrificed and perfused with paraformaldehyde for collection of brain tissue and analysis of hippocampal neurogenesis and associated cell-fate markers (e.g., doublecortin (DCX) and Ki67, see Figure 10), as well as quantitative measures of DREADDs expression within the infected Ce region. We will perfuse the animals for the BrdU experiments that cannot be optimally performed with fresh frozen tissue. The two-month interval was selected, based on our preliminary data (personal communication, [redacted]), to optimize the detection of the number of BrdU expressing mature neurons in the NHP brain. At multiple time-points over the course of the experiment, we will examine the effects of acute Ce activation using CNO. This will be performed prior to, immediately after (before BrdU administration), and 2 months after chronic C21 treatment (before sacrifice). As in Aims 1 & 2 and as outlined in the General Experimental Design section, each animal will undergo testing in the potentially-threatening NEC paradigm in combination with FDG-PET. Testing will be performed with CNO and vehicle 3 weeks apart, using a crossover design. While select Ce neurons will be manipulated in Aim 1, here, testing prior to chronic drug administration will assess the effects of whole-Ce activation. Testing immediately after chronic drug treatment will allow us to examine enhanced effects of long-term Ce activation. Testing occurring 2 months after drug cessation will assess the extent to which the effects of chronic Ce activation are autonomously maintained. We predict increased metabolism within a distributed network that includes Ce and BST that will be associated with increased AT, further enhanced after chronic Ce activation, and maintained 2 months later in the absence of the designer drug. Importantly, we predict that chronic Ce activation will cause structural alterations as evidenced by decreased integrity of the uncinate fasciculus, decreased hippocampal volume, and decreased hippocampal neurogenesis. Based on rodent studies from the McEwen Lab, we also predict that chronic Ce stimulation will result in increased amygdala volume (*118-120*). These studies will reveal, for the first time, the degree to which chronic Ce activation accounts for the distributed, life-long brain changes that affect individuals with stress-related psychopathology.

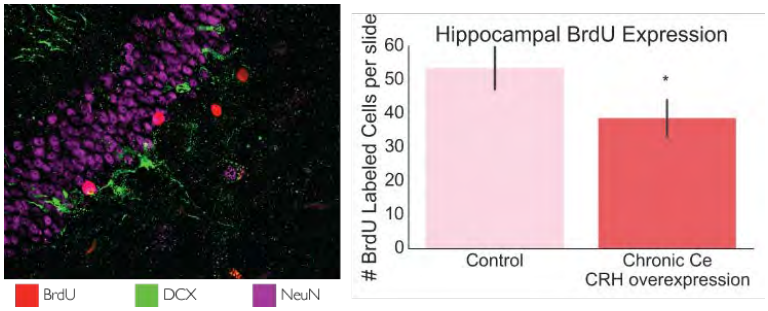


Figure 10. Hippocampal BrdU, doublecortin (DCX) and NeuN expression in young NHPs (*left*). BrdU-labeled cells are reduced after chronic Ce overexpression of CRH (*right*).

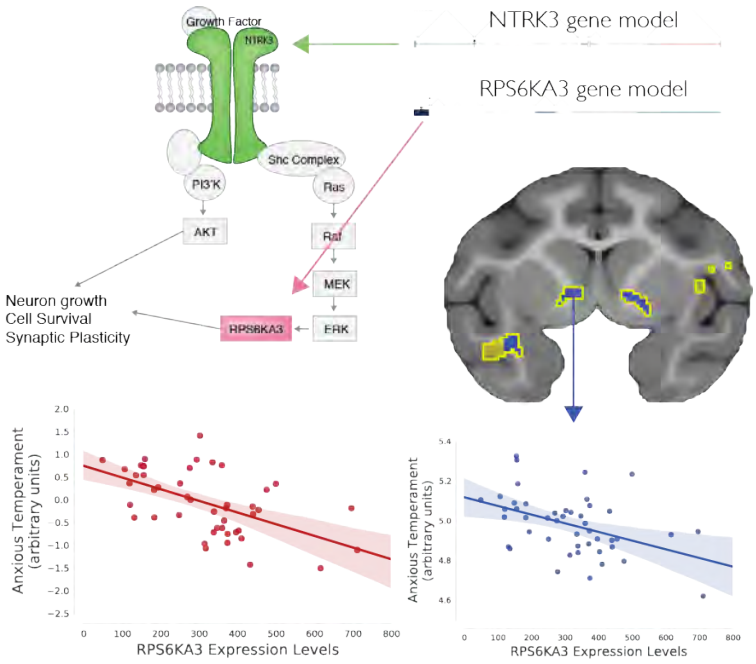


Figure 11. RNA-Seq studies revealed that expression of exons in *NTRK3* (green) and its downstream effector, RPS6KA3 (pink) are inversely associated with AT. Metabolism within the AT-related BST regions was also associated with *RPS6KA3* expression. Plots demonstrating inverse relations between *RPS6KA3* and AT (pink) as well as *RPS6KA3* and BST metabolism (blue) are shown.

AIM 4: *Identify molecular markers of projection-specific anxiety-modulating neurons that will enable development of selective circuit-based treatment approaches.* Studies in Aims 1 & 2 will establish the extent to which the Ce to BST and the OPro/AI to Ce pathways are central to the pathophysiology of AT. Aim 4 is focused on identifying potential molecular markers that are specific to the neurons in these circuits using RNA-Seq. These data will begin to set the stage for the development of new treatments aimed at selectively modulating the activity of specific, anxiety-relevant pathways.

The Kalin[name] collaboration has already produced over a billion RNA-Seq reads from the Ce of 47 animals, putting us at the forefront of NHP transcriptome analyses. Results demonstrated that AT was associated with Ce expression levels of 142 gene features (exons, introns, and junctions) at a p<0.005 threshold. These features represent 119 d ifferent genes, where expression of all or part of the transcript was associated with individual differences in AT. Consistent with our prior work suggesting an important role for Ce neuroplasticity in AT, many of the transcripts that were related to AT encoded molecules involved in mechanisms associated with neuroplasticity and synaptic restructuring. Replicating our microarray findings (*93*), a feature of ribosomal protein S6 kinase, 90kDa, polypeptide 3 (*RPS6KA3*, also known as *RSK2*; Figure 11), as well as a feature of *NTRK3*, negatively predicted individual differences in AT. Interestingly, additional analyses revealed associations among the levels of these transcripts and metabolism throughout the AT-neural network (see Figure 11). We are currently performing similar analyses with respect to the relation between OPro/AI transcripts and AT, and will extend these findings by identifying AT-related transcripts in Ce that are specifically expressed in the subset of neurons that project to BST, and similarly identifying AT-related transcripts in OPro/AI that are specifically expressed in the subset of neurons that project to Ce. This innovative approach has the potential to identify drug targets that are specific to regulatory Ce input, and anxiety-related Ce output.

To accomplish Aim 4, fresh frozen tissue from animals in Aims 1 & 2 will be used for LCM collection of pathway-specific (Ce to BST; OPro/AI to Ce) and adjacent, non-pathway-specific, neurons that originate from the same region. Dual infected pathway-specific neurons will be identified based on their red mCherry fluorescence. Slides will also be labeled with a NeuN antibody visualized with green fluorescence to identify adjacent, non-pathway-specific, neurons (expressing NeuN but *NOT* mCherry) from the Ce (brains from Aim 1) and similarly from OPro/AI (brains from Aim 2). RNA will be extracted for RNA sequencing and expression levels will be compared between pathway-specific and non-pathway-specific neurons. For example, RNA expression levels in pathway-specific neurons harvested from Ce will be compared to RNA expression levels in adjacent non-pathway-specific neurons to identify differences between BST-projecting and non-BST projecting Ce neurons. Our long-time collaborator, Dr. [name] will perform the RNA-Seq analysis at USC. A comparison of the gene expression patterns between pathway-specific and non-pathway-specific neurons, *from the same region*, will enable us to identify hypothetical molecular markers with the potential to selectively modulate neurons originating in Ce that project to BST, or those originating in OPro/AI that project to Ce. We will focus our analyses on cell surface receptors that could provide drugable targets. Based on our data linking Ce *NTRK3* expression to AT, among other genes, we predict that Ce neurons projecting to BST will overexpress the growth factor receptor gene *NTRK3*. We further hypothesize that across all animals in Aim 1 (n=24) *NTRK3* expression levels in BST-projecting Ce neurons will inversely relate to individual differences in AT and AT-related metabolism in the extended amygdala. The strategy of this Aim represents a novel approach for discovering drugs that can prevent the consequences of chronic Ce-hyperactivation. In the unlikely event that Aims 1 & 2 do not reveal selective AT-related pathways, the data from Aim 4 will remain valuable, as it will provide molecular targets for further exploration of the selective function of these pathways.

Additional opportunities in the NHP for assessing translational potential of DREADDs: Our lab has extensive experience on the cutting edge of methods development in NHPs. The availability of DREADDs expressing NHPs is a highly valuable resource and our plan is to maximize the use of these animals and their tissues to further the development of methods and techniques for future chemogenetic NHP studies. Using seed funds

from the UW-Madison Department of Psychiatry ($25,000/year) along with additional resources from the Kalin laboratory, we will, with our collaborators, explore the use of novel tools for *in vivo* and *in vitro* quantification of DREADDs expression as well the consequences of DREADDs activation. We are particularly interested in neuroimaging methods and biomarker development focused on enabling DREADDs technology in humans. Collaborating with [name redacted]), we will explore [^{11}C]-clozapine and other radioligands that may allow for DREADDs visualization with PET imaging (*106, 121-123*). In collaboration with [name redacted] we will continue the development of MRS methods in NHPs to quantify changes in GABA, glutamate, and IP3 levels resulting from DREADDs activation (*124*). Moreover, to examine the precise electrophysiological features of the Ce and OPro/AI neurons studied in Aims 1 & 2, in collaboration [name redacted] will explore the use of *in vivo* (e.g., depth electrodes) and *ex vivo* (e.g., slice electrophysiology) techniques (*125-128*). Additionally, as new DREADDs tools become available from the [name redacted] laboratories, we will attempt to pilot them as part of our collaborative efforts. Findings from these studies, that represent significant advances, will be used to refine the experiments in the current proposal. In addition, we will explore new technology for RNA-Seq of single-neurons, as this field is rapidly evolving. For instance, the [name redacted] laboratory is piloting Drop-Seq techniques (*129*) to enable simultaneous quantification of gene expression in 1,000's of individual cells with individual-cell barcodes. We will pilot these technologies over the course of the project and adjust our strategy accordingly.

Detailed Methods: We have published extensively on many of the methods discussed and featured in this proposal. Due to space limitations we will only provide detailed descriptions of methods that have not been described in our published work. *Research Team*: As Director of the HealthEmotions Research Institute's (HERI) Imaging Laboratory, Chair of the Department of Psychiatry, and affiliate scientist at the Wisconsin National Primate Research Center and Harlow Primate Laboratory, Dr. Kalin is uniquely qualified to direct this study which combines his extensive expertise in monkey models of extreme anxiety with more than 15 years of experience with neuroimaging studies of NHPs. To support the integrated goals of this study, Dr. Kalin has assembled a team of expert collaborators. The team has expertise in DREADDs techniques ([name redacted]), extended amygdala anatomy (Kalin, [name redacted]) and analysis of the markers of hippocampal neurogenesis ([name redacted]). We have developed sophisticated protocols for RT-IMRI-guided targeting of small brain structures ([name redacted] & Kalin). Finally, our expertise in primate anatomy and LCM [name redacted] Kalin) allows for select dissection of BST-projecting Ce neurons (as well as OPro/AI neurons projecting to Ce) for RNA sequencing in collaboration with a leading expert in the field ([name redacted]). *Imaging Parameters and Analysis*: FDG-PET data, anatomical scans and resting state data will be collected and analyzed as described in prior NHP imaging studies (*23-25, 31, 47, 55, 71, 130, 131*). The integrity of white matter pathways connecting Ce with BST (e.g. the stria terminalis and ventral amygdalofugal pathway), and OPro/AI with Ce (e.g., uncinate fasiculus) will be evaluated with DWI collected as in our recent publications (*61, 99, 132*). The RT-IMRI-guided CED surgery methods (*99*) featured throughout the proposal were developed based on the work of our collaborators at the Wisconsin Institute for Medical Research (*99, 133*). Additionally, methods have been published for our behavioral and hormonal assessments of AT, post-mortem histology, euthanasia methods, and other anxiety-related measures. These procedures will be performed in accordance with our standard lab protocols (*22, 25, 31, 46, 47, 53, 55, 71, 93, 131, 134*) that also include measures of emotional reactivity using a broad spectrum of methods established in our laboratory: active defensive behaviors elicited by a staring human intruder; attachment distress elicited by the 'alone' condition of the HIP (*22, 53*); cued fear conditioning (*134*); innate fear induced by snake exposure (*46, 53, 135*); social interactions and defensive behavior in response to a novel, threatening conspecific (*14*); basal and stress-induced pituitary-adrenal activity (i.e. plasma cortisol and ACTH concentrations) (*70, 136*); as well as CSF concentrations of CRH (*32*). Though we are studying pre-adolescent females, we will assess levels of sex hormones and include them as covariates in the analysis when appropriate. We are aware of the potential for the density of sampling to obscure effects of interest, and have effectively dealt with this issue in our previous longitudinal work in NHPs (*22, 24, 70*) that shows this density of sampling does not impact the primary dependent measures. The University of Wisconsin Biological Safety and Institutional Animal Care and Use Committees have approved all of these methods, including the use of AAV and CAV viruses. *Select Methods for LCM and RNA-Seq*: LCM is a well-established method to efficiently isolate specific brain regions or cell types with very little contamination from surrounding tissue. A laser attached to a microscope (Leica LMD6500) will selectively dissect projecting neurons from cryostat-cut tissue sections mounted on slides that are viewed under the scope. We have extensive experience with LCM for capturing neurons from various tissues including Ce, BST, OPro/AI and aHIP. Our recent data demonstrates the viability of the RNA, and in collaboration with the [name redacted] laboratory we are in the process of analyzing RNA-Seq data from LCM-collected Ce neurons from 47 monkeys. Landmarks for Ce and OPro/AI will be determined from alternate 14 µM sections (every 6th section) stained for acetylcholinesterase (AChE), which allows for the identification of the Ce and surrounding structures, as well as the cortical layers within OPro/AI. LCM capture of pathway-specific and non-pathway-specific neurons in Ce (brains from Aim 1) and OPro/AI (brains from Aim 2) will be accomplished using double labeling fluorescence with mCherry and NeuN (Figure 12). Neurons on LCM slides will be identified with a NeuN antibody (MAB377; Millipore, Cambridge, MA) and visualized with a green fluorescently-labeled

secondary antibody (Alexa Fluor 488 goat anti-mouse; ThermoFisher Scientific, Waltham, MA). This allows for rapid neuronal identification with preservation of RNA. [name] is an expert in primate neuroanatomy and has extensive experience with the Leica LMD6500 scope and will perform this technique. Cell boundaries will be delineated and cut by the laser, and automatically deposited into the collection well. RNA-extraction. For LCM of the Ce and OPro/AI neurons, total RNA will be extracted using Direct-zol™ RNA MiniPrep w/ TRI-Reagent® (Zymo Research, Irvine, CA). Testing of 5 different methods found Direct-zol™ to provide the highest yield of miRNA and mRNA. The nucleic acid yield and quality will be assured using an Agilent Bioanalyzer 2100. DNA and miRNA will be extracted and stored for future studies. Transcriptome Sequencing. While we can effectively perform transcriptome analyses on as few as 1-10 neurons as the [name] lab is a leader in single-cell RNA-Seq (*137*), in this proposal we will use pools of approximately 125 pathway-specific and 125 non-pathway-specific neurons collected from each animal from Ce (brains from Aim 1) or OPro/AI (brains from Aim 2). We will use a modification of the SPIA reaction of the NuGEN Ovation RNA-Seq V2 kit for cDNA synthesis, followed by library construction using the NuGEN Rapid no-PCR protocol in a Mondrian microfluidics instrument. This protocol does not use any PCR amplification, and hence does not have as much bias in the measurement of transcripts with low expression levels. Instead we use linear amplification, which amplifies the nucleic acid ~1,000X by the end of library construction. Since the protocol uses random primers for cDNA synthesis it assays non-polyA non-coding RNAs, and provides near perfect 5'→3' read distribution. RNA-Seq libraries will be sequenced to a depth of ~10-25 million SE101 reads using the entire library on Illumina HiSeq DNA sequencers. To demonstrate the reproducibility of the protocol, eight libraries were constructed using 10pg, (= to ~1 cell) of Universal Human Reference (UHR) RNA (Agilent Technologies Inc., 740000) and sequenced. The average r^2 of the 28 pairwise comparisons was 0.59, and ~15,000 transcripts were detected in each library. For 100pg (~10 cells) or 1ng (~100 cells), the average r^2 of the pairwise comparisons was 0.84 and 0.92, respectively. Thus, we have confidence in our ability to obtain quality results from as few as 10 neurons. Read Mapping and Determination of Expression Levels. Data will be analyzed with our own RNA-Seq analysis pipeline, GT-FAR, the next iteration of RseqFlow (*138*). GT-FAR collects and evaluates multiple pre- and post-mapping quality metrics, allowing the elimination of low quality reads in their entirety, or trims out the low quality bases from sequencing library adaptors. Reads are then mapped to the mitochondrial and ribosomal targets and these results are tallied as quality metrics. We then map to the rhesus transcriptome (development version from [name]) and if reads do not map, repeat the mapping allowing for gaps (which discovers unannotated splice events). The remaining reads are then mapped to the rhesus genome, without, and with, gaps, often finding novel exons or transcription units. For RNA-Seq data from Rhesus, we are currently mapping ~70% of reads to the Rhesus transcriptome and genome, and will continue to improve our techniques as new strategies and reference data become available. *Select Methods for Viral Transfection*. For infusion of DREADDs and Cre recombinase viruses we will employ CED RT-IRMI as described in (*99*). The infusion rate will be 1.0 µl/min. The MR visible marker gadobenate dimeglumine (i.e. gadolinium, Gd, MultiHance, Bracco Diagnostics, Cranbury, NJ) will be added to the infusate at a concentration of 0.66 mM. For Aim 1, animals will undergo CED RT-IRMI to infuse into the Ce region (12µl per hemisphere) an rAAV type 5 (rAAV5) that expresses either a Cre-dependent inhibitory DREADDs receptor (AAV5-hSyn-DIO-hM4D(Gi)-mCherry; 10^{12} – 10^{13} vg/ml; n=8) or a Cre-dependent excitatory DREADDs receptor (AAV5-hSyn-DIO-hM3D(Gq)-mCherry; 10^{12} – 10^{13} vg/ml; n=8; vector). DREADDs viral vectors will be purchased from the University of North Carolina (UNC) Vector Core (Chapel Hill, NC). During the same surgery all 16 animals will receive infusions of canine adenovirus (CAV2) containing Cre recombinase (CAV2-Cre; 2 x 10^{12} pp/ml) into BST (12µl per side). The CAV2-Cre virus will be purchased from Plateforme de Vectorologie de Montpellier (Montpellier, France). Sham surgery controls will be injected with CAV2-Cre in combination with a Cre-dependent fluorescent reporter in place of the DREADDs virus (AAV5-hSyn-DIO-mCherry; 10^{12} – 10^{13} vg/ml) (Sham; n=8). For Aim 2, animals will undergo CED RT-IMRI to infuse into the OPro/AI region (24µl per hemisphere) the Cre-dependent inhibitory DREADDs receptor virus (n=8) or the Cre-dependent excitatory DREADDs receptor virus (n=8). During the same surgery all 16 animals will also receive into the Ce region (12µl per hemisphere) the CAV2-Cre virus (n=16). Sham controls will be prepared as in Aim 1. For Aim 3, animals will receive into the Ce region (12µl infusion per hemisphere) an excitatory (non-Cre-

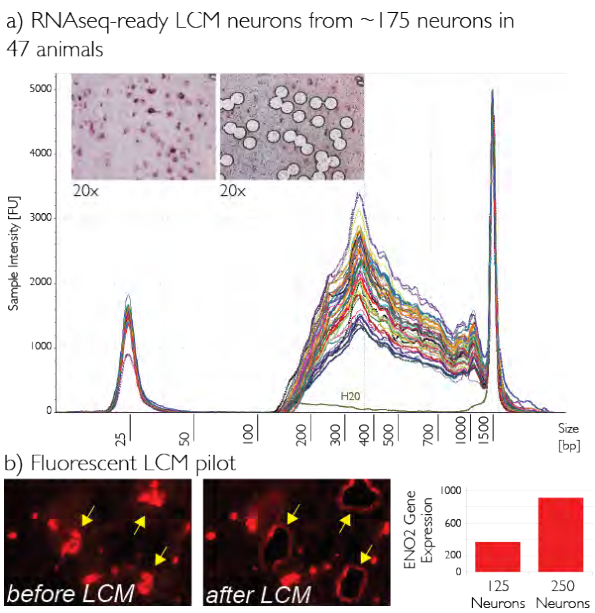


Figure 12. Results from tape station quantification of cDNA from LCM collected mRNA from ~175 Ce neurons in 47 different NHPs demonstrate our ability to reliably and reproducibly use LCM to collect mRNA from fresh frozen brain tissue (*a*). Ce neurons before and after LCM in non-fluorescence (inset, a) and fluorescence (*b*). Neuron specific enolase (ENO2) expression from various numbers of LCM dissected neurons from Ce (*c*).

dependent) DREADDs virus (AAV5-hSyn-hM3D(Gq)-mCherry; 10^{12} – 10^{13} vg/ml; n=12) or will serve as sham controls that will receive AAV5 expressing a fluorescent reporter (n=12). These viruses are also purchased from the UNC Vector Core. CNO and C21 Administration. In Aims 1-3, CNO will be used to acutely activate DREADDs. Based on our pilot studies we will give 5 mg/kg IM 30 minutes prior to testing. The hydrochloride salt of CNO will be made up in a vehicle solution of phosphate-buffered saline (PBS), pH 7.2; CNO and vehicle administration will be counterbalanced with a minimum of 3 weeks between tests. In Aim 3, the dihydrochloride salt of C21 will be administered daily (9 mg/kg IM) in a vehicle solution of PBS, pH 7.2. Comparison animals will receive daily vehicle administration. *Post mortem tissue assessments*: Our laboratory, along with our collaborator[name], has extensive experience using fluorescent and immunohistochemical markers to characterize the anatomical distribution of viral vector transfections, the expression of their constructs in post-mortem tissue, as well as markers of neurogenesis (*99*). In Aims 1 & 2, we will characterize the expression of DREADDs using the conjugated mCherry fluorescence in fresh frozen tissue (e.g., see Figure 3). In Aim 3, fixed tissue will be used to characterize expression of DREADDs, BrdU, DCX, and Ki67. The BrdU, DCX, and Ki67 methods are well established in the [name] laboratory (*140*), and have been used in our NHP tissue (e.g., Figure 10). In Aims 1-3, we will map the distribution of DREADDs expression to ensure accurate infection in the target region, as well as to characterize any expression in adjacent regions. All cell-marker quantification will be performed in single hemisphere, counter-balanced for side, with serial sections throughout the A-P extent of the structure. Hippocampal BrdU-positive cells will be quantified using a modified stereology protocol (*141, 142*). **Analysis, power and sample size. *Power calculations for all studies*:** Our lab has extensive experience using multimodal neuroimaging, and transcriptomic analyses (1, 31, 53, 57, 59, 73, 75, 84, 87). Below is an outline of our analytic strategy. Samples sizes were computed based on power analyses using GPower V 3.1.9.2. Our primary dependent measure in Aims 1, 2 & 3 is change in AT, in Aim 4 our primary dependent measure is gene expression. Because the relations between FDR and statistical power do not rely on the exact number of tests being performed when the number of comparisons is sufficiently large (>30,000) (*143*), we calculated power analyses using α=0.005, the 2-tailed, single-comparison alpha needed to achieve FDR q=0.05 when appropriate. ***Aim 1 & 2: projection specific DREADDs***: Based on our previous lesion studies the effects of DREADDs activation/inhibition on AT are expected to be substantial (η^2=.14). The proposed 8 subjects per group will yield > 85% power to detect similar effects (df=(2,21), calculated in GPower V 3.1.9.2). A 3 X 2 ANOVA will be used to examine the effects of group (inhibitory DREADDs vs. excitatory DREADDs vs. sham surgery) and treatment (CNO vs. Vehicle) on AT and imaging measures. Across group correlational analyses will examine the relationship between changes in AT and changes in neuroimaging measures (e.g., BST metabolism). ***Aim 3: chronic Ce activation with DREADDs***: Based on our previous lesion and chronic viral vector overexpression studies (*53, 99*) the effects of chronic Ce activation with DREADDs on AT are expected to be large (η^2=.14). The proposed 6 subjects per group will yield > 85% power to detect similar effects (df=(3,20), calculated in GPower V 3.1.9.2). After chronic activation with C21, as well as after cessation of C21, we will use 2 X 2 X 2 ANOVAs to examine the effects of group (excitatory DREADDs vs. sham surgery) chronic treatment (C21 vs. vehicle), and acute activation (CNO vs. Vehicle) on AT and imaging measures. At the initial time point, prior to chronic activation, we will use a 2 X 2 ANOVA to examine the effects of group (excitatory DREADDs vs. sham surgery) and acute activation (CNO vs. vehicle) on AT and imaging measures. Across group correlational analyses will examine the relationship between changes in AT and changes in neuroimaging measures (e.g. BST metabolism). To examine the effects of chronic Ce activation on hippocampal neurogenesis, we will use a 2 X 2 ANOVA to examine the effects of group (excitatory DREADDs vs. sham surgery) and chronic treatment (C21 vs. vehicle) on BrdU, DCX, and Ki67 expression. ***Aim 4: RNA-Seq from projection-specific neurons***: Based on our previous microarray (*93, 94*) and RNA-Seq data we expect large differences in expression levels between projection-specific and adjacent, non-projection specific, populations of neurons in neuroplasticity genes (η^2=.14) and robust associations with AT (f^2=.6). Thus, after correcting for multiple comparisons we will have ~75% power to detect differences between sets of neurons (n=24, df=(1,23), calculated in GPower V 3.1.9.2), and ~75% power to detect correlations with AT (n=24, df=22, calculated in GPower V 3.1.9.2). Primary analyses will use a massively univariate approach and standard multivariate regression techniques that will control for potential confounds (e.g., age and sex hormones) and use FDR-techniques (q<.05) to correct for multiple comparisons (*144*). Because we expect a large number of null results in transcripts, we will incorporate the empirical distribution of these null findings into our probability estimation using empirical Bayes techniques (*145*). Transcripts of interest will be examined in relation to variability in AT and brain metabolism. We will perform exploratory pathway and molecular network analyses on relevant RNA-Seq findings using GO enrichment, weighted co-expression gene network, and Ingenuity Pathway Analyses. **All Aims: Multiple comparison corrections for neuroimaging data:** Within each Aim we have primary hypotheses that focus on the role of DREADDs and brain function which are powered similarly to the AT-related analyses, as described above. Follow-up analyses will be performed using robust-regression techniques to attenuate the impact of outliers, and exploratory analyses will use model-free techniques (e.g. ICA/PCA) to further interrogate this rich dataset. In addition to our strong *a priori* predictions, exploratory, whole-brain voxelwise analyses will be performed using an FDR threshold of q<0.05. This approach allows us to correct for multiple voxelwise analyses with moderate power.

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VERTEBRATE ANIMALS

1. DESCRIPTION OF PROCEDURES

To perform these studies, **72 female** rhesus monkeys (*Macaca mulatta*) age 1.5 years, will undergo surgical procedures to infuse viral vectors that express designer receptors exclusively activated by designer drugs (DREADDs) receptors. These receptors enable the ability to selectively excite or inhibit the activity of neurons in response to administration of a designer drug. The effects of this manipulation will be studied using a variety of standard behavioral, hormonal and physiological tests. All animals will be tested repeatedly at baseline and again at various time points after viral vector infusion. Each acute test session will be performed with drug and vehicle on separate occasions, using a counterbalanced crossover design to examine DREADDs-mediated effects on brain, behavior and physiological measures. There will be ~3-weeks between each acute test to ensure enough time for drug washout, and to minimize habituation. The following will be performed: behavioral testing, plasma hormonal sampling, CSF sampling, FDG-PET scan, and MRI. Animals in Aim 3 will be treated with chronic administration of compound 21 (4 months of once daily injections) followed by acute testing to examine the effects of chronic amygdala activation on anxiety-related behavior and brain function. We will also examine the effects of chronic amygdala activation on brain structure and hippocampal neurogenesis.

The following behavioral tests will be performed. Human Intruder Paradigm – The human intruder paradigm is used to assess behavior in 3 different test conditions, alone (AL), no eye contact (NEC) and stare (ST). In the AL condition the animal will be placed in a test cage and behavior will be scored without the presence of a human. During the NEC condition a human intruder enters the test room, never making eye contact with the animal and stands still 2.5 meters from the subject with his/her profile facing the animal. In the ST condition the intruder will enter the test room and maintain eye contact with the animal for the duration of the test. Stranger Conspecific Paradigm - Animals will be temporarily re-housed with a novel animal to evaluate anxiety and arousal during social interaction. During re-housing, behavioral data will be obtained. Animals will then be returned to the home cage with their cage mate. Snake Exposure Test - During the snake exposure test, animals will be placed in a WGTA (Wisconsin General Testing Apparatus) and will be presented with their most preferred food items placed on top of a clear plastic box that contains a stimulus such as a live snake, fake rubber snake, roll of tape or nothing. Each stimulus will be presented in a random order and treat retrieval latency will be recorded as a measure of fear and/or anxiety. Behavior will also be evaluated during each stimulus presentation. Fear conditioning will be performed based on our previous studies (Kalin et al, Physiol Behav: 60, 1043-1046, 1996).

To assess amygdala reactivity with PET, subjects will be injected intravenously with radioactively tagged ^{18}fluoro-deoxyglucose (FDG; up to 10 mCi). During the uptake of this glucose analog, the subjects will be placed alone in a cage for 30 minutes of the NEC condition. At the end of this 30 minute uptake period, subjects will be anesthetized with 15 mg/kg ketamine (IM) and no more than 10 mL blood will be sampled by venipuncture for plasma ACTH and cortisol levels. Animals will then be fitted with an endotracheal tube to deliver 1-5% isoflurane gas anesthesia (IT) during the PET scan while brain metabolic activity will be imaged and evaluated. Within 1 – 2 weeks after PET, high-resolution MRIs will be acquired. These images will be used to assess variation in structural morphometry, to coregister with and align PET data, and to plan surgical procedures. We will examine DREADDs-mediated effects on anxiety-related brain function using FDG-PET and fMRI scans. All MRIs will be acquired within 2 hours while subjects are anesthetized with 15 µg/kg dexmedetomidine (IM) and 15 mg/kg ketamine (IM), which will be repeated as necessary. At least 5 days after MRI, subjects' hormonal response to stress will be evaluated by sampling no more than 10 mL blood by venipuncture before and after relocation to a novel environment. Following this, the subject will be anesthetized with 15 mg/kg ketamine (IM) and no more than 3 mL CSF will be sampled by percutaneous puncture of the cisterna magna for measurement of CRH levels.

DREADDs or Cre recombinase expression will result from MRI-guided inter-cranial injections of a viral vector into the central nucleus of the amygdala (Ce), orbital proisocortex/agranular insula (OPro/AI) or bed nucleus of the stria terminalis (BST). Each infusion will occur at a rate of 1.0 µl/min using volumes specific to the targeted region. For Specific Aim 1 animals will undergo a surgical procedure infusing into the Ce region a recombinant adeno-associated virus type 5 (rAAV5) that expresses either a Cre-recombinase-dependent inhibitory DREADDs receptor (AAV5-hSyn-DIO-hM4D(Gi)-mCherry; N=8) or a Cre-recombinase-dependent excitatory DREADDs receptor (AAV5-hSyn-DIO-hM3D(Gq)-mCherry N=8). During the same surgery all 16 animals will

also receive an infusion into the BST region of a canine adenovirus (CAV) that contains a plasmid that expresses Cre recombinase (CAV2-Cre; N=16). Another set of monkeys will serve as sham surgery controls such that a CAV2-Cre will be injected into the BST in combination with a Cre-recombinase-dependent fluorescent reporter in place of the DREADDs virus (AAV5-hSyn-DIO-mCherry) injected into the Ce (Sham; N=8). For Specific Aim 2 animals will undergo a surgical procedure infusing into the OPro/AI region the Cre-recombinase-dependent inhibitory DREADDs receptor virus (AAV5-hSyn-DIO-hM4D(Gi)-mCherry; N=8) or the Cre-recombinase-dependent excitatory DREADDs receptor virus (AAV5-hSyn-DIO-hM3D(Gq)-mCherry N=8). During the same surgery all 16 animals will also receive an infusion into the Ce region of the Cre recombinase virus (CAV2-Cre; N=16). Another set of monkeys will serve as sham controls such that a CAV2-Cre will be injected into Ce in combination with a Cre-recombinase-dependent fluorescent reporter (AAV5-hSyn-DIO-mCherry) injected into OPro/AI (Sham; N=8). For Specific Aim 3 animals will receive infusions into the BST region with an excitatory (non-Cre-dependent) DREADDs virus (AAV5-hSyn-hM3D(Gq)-mCherry; N=12) or will serve as Sham surgery controls that will receive an AAV5 expressing a fluorescent reporter (Sham; N=12).

Stereotactic MRI-guided Convection enhanced delivery (CED) Infusion Surgery

We will perform AAV and CAV2-Cre infusions with intraoperative MRI guidance. Before each infusion the MR visible marker gadobenate dimeglumine (Gd, MultiHance, Bracco Diagnostics, Cranbury, NJ) will be added to the injectate at a final concentration of 0.66 mM. All animals will be pre-anesthetized with an initial dose of ketamine (up to 20 mg/kg, IM). The following drugs will be administered during the surgical preparation or procedure: atropine sulfate (0.01-0.3 mg/kg, IM or SQ) to depress salivary secretion, and buprenorphine (0.01-0.03 mg/kg, IM or SQ) for analgesia. Mannitol (1.5-2.0 g/kg, IV over 30 minutes), dexamethasone (up to 2 mg/kg, IM or SQ) or other appropriate anti-inflammatory, may be administered to reduce intracranial pressure as needed. Cefazolin (20-25 mg/kg, IM or IV), cephalexin (20-25 mg/kg, PO), or another appropriate antibiotic will be administered as a prophylactic antibiotic. All drug and treatments will be in consultation with veterinary staff and may be adjusted upon their recommendation. Following pre-anesthetization, animals will be fitted with an endotracheal tube and maintained on 5% or less isoflurane depending on vital signs. Lidocaine HCL 2% with epinephrine (2 mg/kg) may be injected subcutaneously to act as a local anesthetic and to control bleeding along the incision. If this dose is not sufficient to control bleeding an additional 1 mg/kg may be administered.

CED infusion uses continuous pressure, which is generated by flow rate, during the injection to push an infusate through the brain tissue. We will combine CED with intraoperative imaging techniques (i.e., real-time positioning during MRI), to identify and place a catheter in the targeted brain region while monitoring the infusions as they occur. Animals may first undergo a baseline MRI scan to accurately plan the target trajectory. Placement of the modified MRI compatible trajectory guide bases will be performed in the [redacted] surgical suite. The surgical procedure is required to install the bases for the guidance system. After the animal is anesthetized, the head will be shaved and catheters may be placed in a peripheral vein, generally the cephalic or saphenous. The animal will then be repositioned in the MRI compatible stereotaxic frame.

After appropriate surgical preparation of the field and using sterile techniques, an incision will be made and the surface of the skull will be exposed. Following MRI-guided stereotaxic coordinates, the brain target area will be identified and bilateral craniotomies will be made in the skull corresponding to the area of projected infusion catheter placement. The bases will then be placed on top of the skull centered over the craniotomy and will be secured in place with up to 3 screws. In addition, dental acrylic or similar material may be used to fill in gaps and increase the stability of the bases. After verifying the position of the guide stem, the skin will be closed over the skull allowing the bases to remain external and accessible for infusions (skin edges will be brought together in front and behind the bases as seen fit to decrease the amount of bone and fascia exposed). The surgery will take approximately 1-2 hours. After the bases have been secured, the animal will be transported to the WIMR MR imaging center under anesthesia determined by veterinary staff. Upon arrival at the MRI, the animal will be placed on the MRI bed and returned to isoflurane anesthesia. MR imaging will determine if the brain infusion catheter is properly placed for accurate trajectory and will continue during infusion.

A saline filled sealed guide tube (or other appropriate MRI-visible liquid) will be inserted into the base and pivoted using a joystick-like device, to locate the proper trajectory based on MR images of the brain. This trajectory will be located prior to inserting any portion of the catheter. Once appropriate trajectory has been determined, the trajectory will be fixed with a locking ring. The guide tube will then be removed and a catheter

will be inserted. Tubing will connect the catheter to a syringe that will be set into an infusion pump. When the selected target position is confirmed, the catheter will be slowly introduced into the brain to the predetermined depth and the position will be confirmed. Special care will be used to avoid partial extraction movement of the catheter while in the brain. After trajectory and depth have been confirmed, infusion will begin. It is expected that this procedure will last several hours.

After the infusion and MRI acquisition is completed, the infusion catheter will be extracted and the animal will be transported to a surgery room or surgical prep area name/id under anesthesia determined by veterinary staff. The bases will be removed, the incision closed in layers, and the animal will be allowed to recover from anesthesia. The animal will be allowed to recover at least 2 months before post-surgical testing will begin.

Assessing Neurogenesis

For Specific Aim 3, to assess the impact of chronic Ce activation on levels of neurogenesis, at the end of chronic compound 21 administration, monkeys will be treated with BrdU. We have chosen a BrdU dosing schedule that is well-tolerated and captures a maximal number of cells in the 'S phase' of mitosis based on previous primate studies. All animals will be briefly sedated with ketamine 10 mg/kg/IM and administered BrdU (100 mg/kg body weight, diluted in saline plus 7 mM NaOH; Sigma, St. Louis, MO, slowly via saphenous vein) every 24 hours x 5 doses. Animals will be allowed to survive 2 months to allow for cell maturation, and to be consistent with extant literature and data obtained from pilot studies at NIMH. Following perfusion, brain tissue will be obtained and processed for BrdU immunohistochemical analysis.

2. JUSTIFICATIONS

Rhesus monkeys serve as an excellent model of fear and anxiety because they have a well-developed prefrontal cortex and extensive neural linkages between the frontal/temporal regions of the neocortex, the extended amygdala and the hippocampus. These are the same neural circuits that subserve the expression of fear and anxiety in humans. Studies of this nature are critical to understanding the mechanisms underlying the development and expression of psychopathology and they cannot be performed in humans. Furthermore, these studies will utilize the vast framework of data that has been generated from the study of this species.

Primates provide the opportunity to examine fearfulness in social situations which is critical to understanding the expression of human anxiety and fear. Work by numerous laboratories, including our own, demonstrates that the rhesus monkey is the most appropriate animal model for these studies. The social and rearing behavior of these animals is quite similar to that of humans. For example, both rhesus monkeys and humans frequently use nonverbal visual cues in assessing and communicating a specific emotional state to a conspecific. Of particular importance, our work has demonstrated considerable behavioral and physiological parallels between monkeys and children with extreme behavioral inhibition and anxious temperament, including HPA axis, CSF CRH measures and functional brain alterations.

3. MINIMIZATION OF PAIN AND DISTRESS

Any discomfort, distress, pain, and injury will be minimized by the appropriate use of anesthetic and analgesic drugs under the direction and supervision of the veterinary staff. The PET procedures will be performed after animals have be given 15 mg/kg ketamine (IM), 0.27mg atropine (IM) and fitted with an endotracheal tube that will deliver 1-5% isofluorane gas anesthesia (IT). Heart rate, respiration and oxygen saturation will be monitored throughout the PET procedure, and body temperature will be maintained using a warm air blanket. CSF sampling will be performed while subjects are anesthetized with 15 mg/kg ketamine (IM). MRIs will be obtained while the animals are anesthetized with 15 µg/kg dexmedetomidine (IM) and 15 mg/kg ketamine (IM), which will be repeated as necessary. Heart rate and oxygen saturation will be monitored throughout the MRI procedure. BrdU injections will be performed while subjects are sedated with 10 mg/kg ketamine (IM). Stereotactic surgery will be performed after animals are pre-anesthetized with 15 mg/kg ketamine (IM) and fitted with and endotracheal tube that will be used to deliver 1-5% isoflurane gas anesthesia (IT) to maintain deep anesthesia throughout the procedure. Heart rate, respiration and oxygen saturation will be monitored throughout the surgical procedure, and body temperature will be maintained using a warm air blanket. Buprenorphine and/or acetaminophen will be administered following surgery as prescribed by veterinary staff to minimize pain.

4. EUTHANASIA

The method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and approved by our institutional animal care and use committee.

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RESOURCE SHARING PLAN

Data overview: The overarching aim of the proposal is to understand how neural pathways within an anxiety-related neural network that includes the central nucleus of the amygdala (Ce), bed nucleus of the stria terminalis (BST) and orbitofrontal cortex (orbital proisocortex/agranular insula; OPro/AI) control the expression of anxiety-related behavior in young female monkeys. To achieve this we will use designer receptors exclusively activated by designer drugs (DREADDs) technology to selectively excite or inhibit these specific neural pathways and assess the impact on the expression of anxiety-related behaviors and the impact of chronic early-life activation of this circuit on functional and structural brain changes associated with stress-related psychopathology. Complete datasets from monkeys (N=72) will include behavioral and endocrine measures, and multimodal imaging (T1, EPI [resting fMRI], FDG-PET, DWI). Data from sacrificed animals will include RNAseq analyses (N=48) of projection-specific, anxiety-modulating neurons and immunohistochemical analysis of hippocampal neurogenesis (N=24). Unanalyzed tissues will be stored for future research. In the remainder of this section, we use the term “summary-level data” to refer to data that has been processed, analyzed, and stored in an appropriate digital format (e.g., spreadsheets, whole-brain imaging maps, or transcript alignment files). Tissue collected from these monkeys will be made available to qualified researchers upon request.

Sharing data: After manuscripts describing these data are accepted for publication, we will make summary level data publicly available in a timely manner. Specifically, we plan to make these data freely available to the research community using standard, robust information technology tools (e.g., wikis). We do not plan to enforce security (authentication/authorization) on summary-level data served through the portal. We plan to integrate our web portal with the UCSC Genomics browser, possibly adding custom tracks to its standard capabilities. We will also investigate integrating the Galaxy project browser on top of the UCSC browser. Probable genes will be annotated and those with sufficient support will be deposited in public databases. For example see http://at.psychiatry.wisc.edu/, an RNAseq database from our collaboration with [redacted] Imaging data will be converted to NIfTI format (http://nifti.nimh.nih.gov/nifti-1) prior to sharing to maximize ease of access. Summary-level data will be accompanied by extensive metadata and other documentation detailing relevant variables and file formats. Identification numbers will link data across modalities (e.g., imaging to endocrine and transcriptomic). Users will be encouraged to acknowledge the source of the data in secondary publications.

Sharing software: We have also developed a set of software tools for image visualization and region of interest drawing. These tools have been freely distributed and are extensively described on a website hosted by UW (see http://brainimaging.waisman.wisc.edu/~oakes/). We will also freely distribute to the research community any newly developed software tools resulting from this research on our laboratory websites. We will only make software and source code available to other scientists at non-profit institutions. Specific components of software we develop may be incorporated into enhanced products for commercialization. These procedures are in accordance with policies at UW and the Wisconsin Alumni Research Foundation (WARF), the entity that handles intellectual property at UW.

Other: In addition, we will freely share software, data analytic scripts for physiological and image data processing, research protocols, and other details of experimental procedures in response to specific requests from other scientists. All data will be maintained after the end of the award period, in accordance with NIH rules.