RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF MENTAL HEALTH

Federal Award Date: 07/15/2015

Grant Number: 1R01MH107563-01
FAIN: R01MH107563

Principal Investigator(s):
Ned H Kalin, MD

Project Title: 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

BRENDA A. EGAN
Interim Managing Officer
The Board of Regents of the University of Wisconsi
Research & Sponsored Programs
21 N. Park Street, Suite 6401
Madison, WI 537151218

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

Period Of Performance:
Budget Period: 08/01/2015 – 04/30/2016
Project Period: 08/01/2015 – 04/30/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $764,596 (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 R01MH107563. 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,

Dianna N Bailey
Grants Management Officer
NATIONAL INSTITUTE OF MENTAL HEALTH

Additional information follows

SECTION I – AWARD DATA – 1R01MH107563-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$127,582
Fringe Benefits	$42,458
Supplies	$7,478
Other Costs	$322,218
Federal Direct Costs	$499,736
Federal F&A Costs	$264,860
Approved Budget	$764,596
Total Amount of Federal Funds Obligated (Federal Share)	$764,596
TOTAL FEDERAL AWARD AMOUNT	$764,596
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$764,596

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$764,596	$764,596
2	$683,846	$683,846
3	$682,925	$682,925
4	$677,887	$677,887
5	$677,034	$677,034

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:	RMH107563A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2015

IC	CAN	2015	2016	2017	2018	2019
MH	8472591	$764,596	$683,846	$682,925	$677,887	$677,034

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

NIH Administrative Data:
PCC: B4-TBAF / **OC**: 414A / **Released**: [redacted] 07/08/2015
Award Processed: 06/15/2015 11:31:44 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01MH107563-01

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

SECTION III – TERMS AND CONDITIONS – 1R01MH107563-01

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

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget

period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

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

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

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) R01MH107563. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

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

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

Treatment of Program Income:
Additional Costs

SECTION IV – MH Special Terms and Conditions – 1R01MH107563-01

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

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 **4/30/16** end date. Subsequent budget periods will begin on **5/1**, 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. Additional time may be requested at the end of the project period if needed.

PARTICIPANT RECRUITMENT - MILESTONES:
Future NIMH support for this study is contingent upon adequate participant recruitment based on projected milestones as approved in the Recruitment Milestone Reporting system (RMR) on 3/31/2015**.** It is expected that **50** of the **180** total projected participants will be recruited by ***4/1/2016***. This tri-yearly recruitment report should be submitted electronically to NIMH after each milestone period of April 1, August 1 and December 1 at http://wwwapps.nimh.nih.gov/rmr/displayHome.action. In the event that actual recruitment falls significantly below projected milestones, NIMH may consider withholding future support and/or negotiating an orderly phase-out of this study. Information regarding the NIMH Policy for the Recruitment of Participants in Clinical Research is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-MH-05-013.html.

ADMINISTRATIVE REDUCTION:
In order to meet Institute program objectives within Fiscal Year 2015 budget constraints, future years recommended levels of support for this grant have been reduced by 10**%.**

CONSORTIUM/CONTRACTUAL COSTS:
The year -05 commitment includes funds for consortium activity with University of Southern California. Each consortium is to be established and administered in accordance with the NIH Grants Policy Statement dated March 31, 2015. No foreign performance site may be added to this project without the written prior approval of the National Institute of Mental Health.

RESOURCE / DATA SHARING PLAN:
The grantee is required to comply with the Genomic Data Sharing Policy, which is stated in this Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-124.html

STAFF CONTACTS

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

Grants Management Specialist: Jackie Chia
Email: Jackie.Chia@nih.gov **Phone**: 301-443-1341 **Fax**: 301-480-1956

Program Official: Holly A. Garriock
Email: holly.garriock@nih.gov **Phone**: 301-443-9230 **Fax**: 301-480-4415

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01MH107563-01

INSTITUTION: UNIVERSITY OF WISCONSIN-MADISON

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$127,582	$109,339	$233,010	$238,311	$238,220
Fringe Benefits	$42,458	$36,252	$80,403	$82,296	$82,264
Supplies	$7,478	$9,430	$9,551	$13,533	$11,003
Travel Costs			$4,500	$4,500	$4,500
Other Costs	$322,218	$291,937	$118,892	$104,423	$52,650

Consortium/Contractual Cost					$69,169
TOTAL FEDERAL DC	$499,736	$446,958	$446,356	$443,063	$457,806
TOTAL FEDERAL F&A	$264,860	$236,888	$236,569	$234,824	$219,228
TOTAL COST	$764,596	$683,846	$682,925	$677,887	$677,034

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	53%	53%	53%	53%	53%
F&A Cost Base 1	$499,736	$446,958	$446,356	$443,064	$413,637
F&A Costs 1	$264,860	$236,888	$236,569	$234,824	$219,228

PI: **Kalin, Ned H**	Title: 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	
Received: 10/03/2014	FOA: PA13-302	Council: 05/2015
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 MH107563-01	Dual: HD	Accession Number: 3741794
IPF: 578503	Organization: UNIVERSITY OF WISCONSIN-MADISON	
Former Number:	Department: PSYCHIATRY	
IRG/SRG: NPAS	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 499,736 Year 2: 496,621 Year 3: 495,955 Year 4: 492,296 Year 5: 478,990	Animals: Y Humans: Y Clinical Trial: N Current HS Code: [redacted]evaluative 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]names - excluded by agreement	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	Other (Specify)-Psychologist
	The Board of Regents of the University of Wisconsin System	Other (Specify)-Statistian
	The Board of Regents of the University of Wisconsin System	Other (Specify)-Scientist
	University of Southern California	Other (Specify)-Subcontract PI
	The Board of Regents of the University of Wisconsin System	Other (Specify)-OSC
	University of Southern California	Co-Investigator
	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
	The Board of Regents of the University of Wisconsin	Other (Specify)-OSC

names - excluded by agreement	System	
	The Board of Regents of the University of Wisconsin System	Other (Specify)-OSC
	National Institutes of Health	Other (Specify)-OSC
	Harvard University	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

b. Agency Routing Number

2. DATE SUBMITTED 2014-10-03

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: 6082626565 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*
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

12. PROPOSED PROJECT
Start Date* 07/01/2015
Ending Date* 06/30/2020

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*	$3,787,237.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,787,237.00
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*: Interim 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*: baegan@rsp.wisc.edu

Signature of Authorized Representative*
BRENDA A EGAN

Date Signed*
10/03/2014

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Risk_coverletter1017091965.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

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 00005399

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*	Risk_ProjectSummary_final1017091718.pdf
8. Project Narrative*	Risk_Project_Narrative_final1017091717.pdf
9. Bibliography & References Cited	Risk_Bibliography_FINALFRIDAY1017091917.pdf
10.Facilities & Other Resources	Risk_Facilities_Other_Resources_final1017091839.pdf
11.Equipment	Risk_Equipment_final1017091840.pdf

Project Summary
Persistent and high levels of sustained anxiety during childhood are a strong predictor of the development of anxiety and depressive disorders during adolescence. This is particularly relevant to females because after puberty girls are twice as likely to develop these disorders. The goal of this proposal is to understand the biological mechanisms of sustained anxiety in highly anxious girls, how it changes over time, and how it can transform into psychopathology. Capitalizing on our unique experience with studies of anxiety in both human and nonhuman primates, we will use a translational neuroscience approach to understand the neurobiology of sustained anxiety in highly anxious girls and young anxious female rhesus monkeys. Neuroimaging studies will focus on the bed nucleus of the stria terminalis (BST) because it is thought to be involved in sustained anxiety and prolonged threat preparedness. Similar paradigms will be used in humans and monkeys to: 1) characterize developmental trajectories of brain function in highly anxious girls; 2) examine the relevance of altered BST function in relation to the onset of anxiety and depressive disorders; 3) test the causal role of the BST in anxiety as young anxious female monkeys mature into adolescence; and 4) define BST molecular alterations that are linked to altered BST function and sustained anxiety. Although sustained anxiety responses can be adaptive, many patients with stress-related psychopathology experience extreme levels of maladaptive sustained anxiety, especially under conditions of uncertainty. To understand the neural underpinnings of sustained anxiety in anxious girls across the transition to adolescence, 170 highly anxious girls will be followed from age 10/11 to age 13/14 with clinical assessments, multimodal neuroimaging, and behavioral and hormonal measures of sustained anxiety. Based on previous work, it is expected that 40% of these girls will maintain high levels of anxiety into adolescence, with up to half of these stably anxious girls developing *bona fide* anxiety and/or depressive disorders. Parallel functional neuroimaging tasks will be used with anxious girls and anxious female nonhuman primates to assess brain activation associated with sustained anxiety during exposure to prolonged and uncertain threat. Mechanistic studies in young anxious female monkeys will use precise MRI-guided lesions of BST neurons to test the causal role of the BST in anxious behavioral and to identify, for the first time in primates, regions that are functionally modulated by BST input. Finally, BST neurons will be harvested from monkey brains using laser capture microdissection, and RNA deep sequencing will be used to link variations in gene expression to anxiety severity and BST metabolism. These translational studies provide the opportunity to test hypotheses about the clinical relevance and causal role of BST function. In addition, examining the molecular composition of BST neurons provides an invaluable opportunity to identify novel molecular mechanisms that contribute to the at-risk child phenotype. This combination of modalities and methods has high translational potential for developing novel, BST-focused, anti-anxiety treatments.

Project Narrative

Persistent and high levels of sustained anxiety during childhood are a risk factor for the development of anxiety and depressive disorders during adolescence, especially in girls who are twice as likely to develop these disorders after puberty. The proposed studies use a translational approach to study brain development in anxious girls and anxious female monkeys to understand the biological mechanisms that support sustained anxiety, how they change with development, and their role in the emergence of psychopathology. These studies will allow for the examination of markers of risk and resilience, and may inform the development of novel treatments to reduce the suffering of girls with anxiety and depressive disorders.

Facilities and Other Resources: University of Wisconsin-Madison

Scientific Environment:
The University of Wisconsin has a long and recognized history of research with human and 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 and Psychology, the HealthEmotions Institute 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 Medical Physics Cyclotron Laboratory. 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.

MRI Facilities:
The [redacted] is located [redacted] the Department of Psychiatry at the University of Wisconsin - Madison. It houses a GE 3T x750 research scanner for structural and functional brain imaging, an MRI simulator room, an MRI preparation room for pre- and postscan behavioral measures, image processing areas with extensive computing facilities, and a conference room and office space. Dr. Kalin directs the neuroimaging laboratory and will make sure that scanning times are available that are appropriate for school age children (after school, early evenings, and weekends).

We primarily use the manufacturer's standard software for acquisition of MRI and fMRI data. Stimulus presentation can be via a back-projection system, or using an advanced fiber optic goggle system (Avotec). MRI auditory stimuli are presented using a pneumatic headphone system (Avotec). All auditory stimuli are presented through a digital equalizer that is optimized for tone and clarity.

Stabilizing patients to minimize head movement is critical for MRI studies. The lab has two head stabilizing methods: vacuum pillow and foam inserts.

Collection of peripheral physiological measures in the MRI is done using the Biopac MP150 system. The MP150 system provides high resolution (16 bit), variable sample rates for analog and calculation channels, 16 analog inputs and two analog outputs, digital I/O lines (automatically control other TTL level equipment), and\ 16 online calculation channels. The MP150 System provides high-speed acquisition (400 kHz aggregate), and Ethernet connectivity. Controlling the Biopac is the software, AcqKnowledge. This software is an interactive program that lets lab members instantly view, measure, analyze, and transform the incoming data. The ability to collect both cardiac and respiration activity (sampling rate of 1000 Hz) is possible through the manufacturers standard software.

To acclimate patients to the unique environment of the MRI scanner the lab houses a simulator room. This room has a mock MRI scanner, another Avotec fiber optic goggle system, and auditory system with two control computers. This room is used to introduce subjects to the identical experimental procedures that they will experience in the actual scanner. MRI simulation is done to ensure subject comfort and data quality, and to allow subjects to repeatedly practice keeping still in the scanner prior to data collection. The proposed study will benefit greatly from this procedure, as it will facilitate the collection high quality imaging data in children and adolescents.

Laboratory:
The UW Department of [redacted] 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. All hormonal assays will be performed at this facility.

Radiochemistry Laboratory:
The [redacted] houses the cyclotron and control rooms, 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. The location of state of the art laboratory and office space. 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 location . 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.

Computing Facilities:
The location network is located in a temperature controlled secure location at the location All servers and SAN equipment are on generator-backed circuits, with UPS to provide power during cutovers. Authentication is provided by proprietary info

The authentication and file server is 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 proprietary info

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 in-house 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 location. These include Macs and PCs for accelerator control, HPLC, GC, and nuclear spectroscopy system. There are also Macs at the location 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 UW Departments of location

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

Facilities and Other Resources: University of Southern California

Computer:
The Genomic Psychiatry Cohort maintains all de-identified demographic, phenotypic, and genetic data for all cohort members, including those ascertained by all sites nationally and internationally, in a SQL-based proprietary database ("Progeny"). Progeny is a clinical data management software that is HIPAA compliant with strong security down to the individual user level. The GPC database server is stored and maintained in-house along with ongoing contracted support from Progeny software developers. Progeny is accessible from anywhere using the password protected web-based client interface, and data may be easily exported to various formats allowing thorough data analysis.

The Center for Genomic Psychiatry has 21 IBM compatible PCs running 64-bit Vista/Win7 (8 of these are dedicated to laboratory equipment), two Windows 2003 Servers with RAID5 arrays, and one Dell PowerEdge R710s with 192 GB of RAM and 12 TB disk running VMWare ESXi. We also have a two Silicon Mechanics StorServs with ~400 TBs of useable disk space (including the 3 JBODs) running Solaris ZFS. On each floor of the location there is available a shared computing facility, which is equipped with several Dell workstation PCs and Macintosh G5 computers, a color laser printer, a Xerox copy machine and a Canon fax machine. All computers in the PI's lab and the institute are directly connected to high-speed internet, and a 10 Gb fiber connection links location.

USC has a Linux cluster configuration (HPCC) that is ranked as the 5th fastest academic supercomputer in the USA and 17th fastest academic supercomputer worldwide, and 63rd fastest of all supercomputers worldwide (Spring 2011). The 1,988-node (each with 8-12 cores), 10-gigabit backbone benchmarked at 126.4 teraflops. The Center co-owns a dedicated "condo" of 122 nodes (1,308 cores). The Center laboratory solely owns a 40 core server with 1 TB of RAM for high-memory compute jobs. Dr. name a professor and the associate chair for research in Psychiatry, leads a consortium of USC researchers who purchased a high-performance 1.2 PB (~800 TB usable) DDN 10K-e disk array in 2011. ~400 TB of this space is owned by the name laboratory and further expansion of this device to 2.4 PB (raw) can be accommodated by upgrading the head node to a 12K-e (available now), if needed.

Laboratory:
The central laboratory available to the GPC occupies location and consists of one main room with 6 separate sub laboratories. In addition, the PI has access to two shared cold-rooms, several common facility rooms, a liquid nitrogen storage room, and one

autoclave/dishwasher room in [redacted]. An organized research unit of the Keck School of Medicine [redacted] is a state-of-the-art facility housed [redacted]

Office:
The Center for Genomic Psychiatry within the Department of Psychiatry and Behavioral Sciences is located on the USC [redacted] space, 30 offices, 12 administrative support workstations, 2 large workrooms (copy/filing/mail), a reception/waiting area, break room, and a partitionable conference room that seats approximately 25 people and contains two audio visual systems. Private office space, meeting rooms, and workstations are provided by the department for use by all USC project personnel.

Equipment: University of Wisconsin-Madison

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. MicroPET and Accelerator
The [redacted] 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 portable Concord Microsystems microPET-P4, which is housed at the [redacted] and dedicated fully to this research. This microPET has a large enough field of view (FOV) to allow for single position acquisition of an entire rhesus brain. The expected absolute sensitivity of 250 keV is 500 cps/mCi. Several data reconstruction algorithms are available, including filtered back projection (FBP) and ordered subset expectation maximization (OSEM) modified for 3D data sets. The calculated image resolution with FBP in the central FOV is 2 x 2 x 2 mm.

3. Microscope
We have a Leica LMD6500 Laser Microdissection System housed at the UW [redacted]. 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.

4. Hormonal assay
[redacted] has a fully equipped 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 Victor2 plate luminometer and a gamma counter for radioimmunoassays.

Equipment: University of Southern California (USC)

1. Center for Genomic Psychiatry Laboratory
Three Illumina HiSeq2500 DNA sequencers; 1/3 Ownership of an Illumina Genome Analyzer IIx (GAIIx) DNA sequencers with paired-end module; Two Illumina cBots; One NuGEN Microfludic Sample Prep System; 1 Caliper LabChip XT Nucleic Acid Size Selection and Collection System; 1/3 Ownership of an Illumina BeadExpress Reader; 1 Hamilton STARlet with both 8 and 96 channel pipettors and integrated Trobot PCR machine; 1 TECAN 8 channel Genosys robotic workstation; 1 Apogent Hydra 96 channel robotic pipettor; 1 AutoGenFlex STAR DNA Extraction System with sample Tracking Software; 2 QiaGen QiaCube Extraction Robots; 1 BioMicroLab XL20 Tube Handler robot with 2D Scanner and Analytical Balance; 1 BioMicroLab SampleScan Mini; 1 Agilent 2200 TapeStation Nucleic Acid System; 1 Agilent 2100 BioAnalyzer; 1 Caliper LabChip DS High Throughput UV-VIS Spectrophotometer; 1 TECAN Ultra Fluorescent Polarimeter and plate reader; 1 TETRA Thermal Cyclers with 4-384 well plates (MJ Research); 2 DYAD Thermal Cyclers with 2-384 well plates (MJ Research); 2 thermal Cyclers for 96-well plates (MJ research); 1 Sanyo MCO-18AIC(UV) CO_2 incubator (Sanyo), 1 Zeiss inverted microscope (Zeiss), SpeedVac SC110A (Savant), Termomixer R (Eppendorf); multiple gel boxes and power supplies; 1 Beckman Ultracentrifuge, centrifuges, balances, water baths, -80^{o}C freezers, incubators, ovens, microfuges.

2. The [redacted] at USC
provides many state-of-art, shared facilities that are available to the [redacted] lab, including a single-channel NanoDrop Spectrophotometer, gamma counters, scintillation counters, Beckman high-speed and ultra-speed centrifuges, Phosphoimager (Typhon 860); Biorad ChemiDoc systems, Leica two-photon microscopes, Zeiss LS510-meta confocal microscope, JEOL electron microscope, Aviv CD spectrophotometer, Thermo-Finnegan mass-spectrometers with HPLCs, automated DNA sequencers, oligonucleotide/peptide synthesizers, protein sequencing facility, an 700MHZ NMR spectrometer, an X-ray diffractor, and etc.

3. USC Genomics Laboratory:
Located [redacted], the equipment of the Genomics laboratory is available for use by the [redacted] lab staff. This equipment includes, one Illimina HiSeq 2000 DNA sequencer, three Illumina GAIIx DNA sequencers, all with a paired-end modules (1/3 of one of these is owned by [redacted] lab). The Genomics laboratory also has a full Illumina BeadLab System and a BeadExpress Reader. This system is a production SNP genotyping/expression platform that includes automation equipment (including two Tecan

Genesis robotic workstations), Laboratory Information Management System (LIMS), BeadArray Readers, BeadStudio data analysis software, hardware and accessories for the generation of millions of genotypes per day or hundreds of thousands of gene expression profiles. The Genomics Core Facility also has the following additional equipment available: Bio-Rad Experion, several liquid handling robots including a Beckman MultiMek, a Qiagen BioRobot 3000, a Qiagen Rapidplate with Twister II, and a Tomtec Quadra 384. They also have Fluidigm BioMark MX/HX real-time analysis system with 4 FC1 thermal cyclers, 8 IFC controllers, 4 HX controllers, 2 MX controllers and 2 Access Array controllers for loading and unloading of array chips for genome partitioning. In addition, the cores utilize an ABI7900HT Sequence Detection System for single SNP genotyping, an ABI 3730xl DNA Analyzer for DNA sequencing and microsatellite analysis, thermal cyclers (MWG Biotech and ABI), balances, centrifuges (microcentrifuges and table-top), gel apparatus and power supplies, fluorometer, water baths, and computer workstations to assist in data generation and collection.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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 E-Mail*: NKALIN@WISC.EDU

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

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

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	Kalin_bio1017091712.pdf
Attach Current & Pending Support:	

secondary names and identifiers

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kalin, Ned H.	Hedberg Professor and Chairman, Department of Psychiatry, University of Wisconsin School of Medicine and Public Health
eRA COMMONS USER NAME (credential, e.g., agency login) username	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Pennsylvania State University, PA	B.S.	1972	Pre-Medicine
Jefferson Medical College, PA	M.D.	1976	Medicine

A. Personal Statement

I have been an NIH-funded PI for more than 25 continuous years and have published over 200 peer-reviewed journal articles related to the adaptive and maladaptive expression of emotion and anxiety. My work has focused on the development of nonhuman primate models to understand mechanisms underlying the development of anxiety and affective disorders. Our findings have identified and validated a young monkey phenotype that maps on to the early-risk phenotype in children, and in monkeys we have defined the underlying neural circuit using lesioning methods and imaging strategies. We have extensive experience characterizing this anxious temperament in over 600 monkeys using a combination of behavioral and endocrine measures and in vivo imaging strategies including magnetic resonance imaging and positron emission tomography. I have also integrated my preclinical studies with human functional imaging studies in patients with anxiety and depression, and children with anxiety disorders, providing important insights into the factors and neural circuitry underlying the risk to develop anxiety disorders and depression. My previous work with both human and nonhuman primate models of anxiety make me uniquely qualified to accomplish the goals of this translational study, with the ultimate goal of developing new, neurally-informed treatment approaches to reduce the risk to develop anxiety and depressive disorders.

B. Positions and Honors

Positions and Employment

1976-1979 Resident, Department of Psychiatry, University of Wisconsin Medical School – Madison
1979-1981 Staff Psychiatrist, Clinical Neuropharmacology Branch, NIMH, Bethesda, MD
1981-1983 Assistant Professor of Psychiatry, University of Wisconsin-Madison
1981-1987 Director, Psychiatry Special Diagnostic and Treatment Unit, VA Hospital, Madison
1984-1986 Associate Professor of Psychiatry, University of Wisconsin-Madison
1981-1991 Senior Staff Psychiatrist, VA Hospital, Madison
1981-present Affiliate Scientist, Wisconsin Regional Primate Center; Research Psychiatrist, University Wisconsin Primate Laboratory
1986-present Professor of Psychiatry, University of Wisconsin-Madison
1991-present Professor of Psychology, University of Wisconsin-Madison
1991-present Hedberg Professor and Chair, Department of Psychiatry, University of Wisconsin-Madison
1996-present Director of HealthEmotions Research Institute

Other Experience and Professional Memberships

Co-Editor-in-Chief, Psychoneuroendocrinology
Past President, International Society of Psychoneuroendocrinology
Member, National Advisory Mental Health Council, National Institutes of Health (through Sept. 2007)
Fellow, American Association for the Advancement of Science

President, Society of Biological Psychiatry

Honors

1985 A.E. Bennett Award for basic research in biological psychiatry
1996 Distinguished Alumni, Jefferson Medical College Department of Psychiatry
2001 Fellow, American College of Neuropsychopharmacology
2001 Fellow American College of Psychiatry
2002 Wisconsin NAMI Research Award
2005 Edward A. Strecker, M.D. Award
2007 American College of Psychiatrists, Award for Research in Mood Disorders
2007 Gerald Klerman Senior Investigator Award for Psychiatric Research –DBSA
2009 – 2010 Finance and Budget Committee, American Psychiatric Association
2010 Harold Lawn Memorial Lecture, University of Minnesota
2012 Distinguished Psychiatry Lecturer, American Psychiatric Association
2012 American Association for the Advancement of Science (AAAS) Fellow
2012 – 2016 Scientific Council, Anxiety and Depression Association of America

C. Selected Peer-reviewed Publications (Selected from over 195 peer-reviewed publications)

Most relevant to the current application

1. Kalin NH, Shelton SE. Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. Science 243:1718-1721, 1989.
2. Kalin NH, Shelton SE, Davidson RJ. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Journal of Neuroscience 24:5506-5515, 2004.
3. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH. Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS ONE, 3:e2570, 2008. PMC2430534.
4. Rogers J, Shelton SE, Shelledy W, Garcia R, Kalin NH. Genetic influences on behavioral inhibition and anxiety in juvenile rhesus macaques. Genes, Brain, and Behavior 7:463-469, 2008. PMC2785008.
5. Oler JA, Fox AS, Shelton SE, Rogers J, Dyer TD, Davidson RJ, Shelledy W, Oakes TR, Blangero J, Kalin NH. Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature 466:864-868, 2010. PMC2998538
6. Essex MJ, Klein MH, Slattery MJ, Goldsmith HH, Kalin NH. Early risk factors and developmental pathways to chronic high inhibition and social anxiety disorder in adolescence. Am J Psychiatry. 2010 Jan;167 (1):40-6. PMC2806488.
7. Fox AS, Oler JA, Shelton SE, Nanda SA, Davidson RJ, Roseboom PH, Kalin NH. Central amygdala nucleus (Ce) gene expression linked to increased trait-like Ce metabolism and anxious temperament in young primates. Proc Natl Acad Sci U S A. 109:18108-18113, 2012 PMC3497741.
8. Rogers J, Raveendran M, Fawcett GL, Fox AS, Shelton SE, Oler JA, Cheverud J, Muzny DM, Gibbs RA, Davidson RJ, Kalin NH. CRHR1 genotypes, neural circuits and the diathesis for anxiety and depression. Mol Psychiatry. 18:700-707, 2013. PMC3663915.
9. Shackman AJ, Fox AS, Oler JA, Shelton SE, Davidson RJ, Kalin NH. Neural mechanisms underlying heterogeneity in the presentation of anxious temperament. Proc Natl Acad Sci U S A. 110:6145-50, 2013. PMC3625282.
10. pending publication [redacted] In press. pending publication [redacted]

Additional recent publications of importance to the field (in chronological order)

1. Nitschke JB, Sarinopoulos I, Oathes DJ, Johnstone T, Whalen PJ, Davidson RJ, Kalin NH. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. Am J Psychiatry. 166:302-10, 2009. PMC2804441.

2. Oler JA, Fox AS, Shelton SE, Christian TB, Murali D, Oakes TR, Davidson RJ, Kalin NH. Serotonin transporter availability in the amygdala and bed nucleus of the stria terminalis predicts anxious temperament and brain glucose metabolic activity. J Neurosci. 29:9961-9966. 2009. PMC2756094.
3. Christian BT, Fox AS, Oler JA, Vandehey NT, Murali D, Rogers J, Oakes TR, Shelton SE, Davidson RJ, Kalin NH. Serotonin transporter binding and genotype in the nonhuman primate brain using [C-11]DASB PET. Neuroimage. 47:1230-6, 2009. PMC2798593.
4. Adluru N, Zhang H, Fox AS, Shelton SE, Ennis CM, Bartosic AM, Oler JA, Tromp do PM, Zakszewski E, Gee JC, Kalin NH, Alexander AL. A diffusion tensor brain template for Rhesus Macaques. Neuroimage. 59:306-318. 2012. PMC3195880.
5. Kalin NH, Shelton SE, Fox AS, Rogers J, Oakes TR, Davidson RJ. The serotonin transporter genotype is associated with intermediate brain phenotypes that depend on the context of eliciting stressor. Mol Psychiatry. 13:1021-1027, 2008. PMC2785009.

D. Research Support

Ongoing Research Support

P50MH100031 (PI: Davidson, Project 1 Director: Kalin) 09/01/13-08/31/18
NIH/NIMH
Early Neurodevelopmental Origins of Anxiety
Early adversity can have a harmful influence on the development of an extreme emotional disposition termed anxious temperament. This proposal, which integrates advanced multimodal imaging and molecular methods in developing primates exposed to an adversity manipulation, has the potential to yield new mechanistic insights and set the stage for developing novel neuroscientifically-informed interventions.

R01MH046729 (PI: Kalin) 07/01/12-06/30/17
NIH/NIMH
Development and Regulation of Emotion in Primates
In children, extreme behavioral inhibition is associated with an increase risk to develop anxiety disorders and depression. The proposed experiments will use a non-human primate model of behavioral inhibition to identify the roles of stress-related and neuroplasticity-related systems within the amygdala as developmental mechanisms that may increase or decrease, respectively, the childhood risk to develop anxiety and depression.

R21MH092581-01A1 (Kalin) 04/19/12-01/31/15 (NCE)
NIH/NIMH
Brain Mechanisms Underlying Childhood Generalized Anxiety Disorder
The proposed studies are aimed at understanding the brain alterations associated with childhood generalized anxiety disorder (GAD) so that early diagnosis can be improved and more effective interventions can be developed. We aim to build on our work in young nonhuman primates by identifying intermediate brain phenotypes that are linked to early human childhood GAD.

2R01 MH081884-05A1 (Kalin) 04/16/13-03/31/18
NIH/NIMH
Brain Mechanisms Mediating Genetic Risk for Anxiety and Depression
These studies will use an innovative combination of brain imaging and state-of-the-art molecular genetics methods in young monkeys to identify the molecular alterations that occur in the brain systems underlying anxious temperament.

Completed Research Support

P50 MH084051 (PI: Davidson, Project 1 Director: Kalin) 09/01/08-05/31/13
NIH/NIMH

Neurobehavioral Bases of Emotion Regulation and Dysregulation in Adolescence
This center employs an affective neuroscience perspective to further understanding of the underlying neural and behavioral bases of risk for anxiety and mood disorders (internalizing disorders) in adolescence. Project I uses an extensively validated non-human primate model with imaging and molecular methods to understand the neural circuits and molecular mechanisms underlying adolescent vulnerability to develop stress-induced psychopathology.

1R21MH091550 (PI: Kalin) 12/01/10-11/30/12
NIH/NIMH
Combining mouse and monkey models to understand human risk for psychopathology
The long-term goal of this work is to understand how extreme behavioral inhibition as a temperamental disposition that serves as an early vulnerability marker for the development of anxiety disorders and depression. The proposed studies will identify novel genes systems that mediate the extreme temperament by combining molecular studies on our adolescent mouse and monkey models of behavioral inhibition.

The focus of this work is to study postmortem tissue from the brains of normals, major depressives and major depressives with psychosis. The emphasis is on identifying changes in the corticotropin-releasing factor system (CRF) in amygdala and temporal cortical tissue. In addition, other neurotransmitters systems that have been implicated in stress, depression and suicide will also be examined.

R01 MH046729 (PI: Kalin) 08/15/05-06/30/11
NIMH
Development and Regulation of Emotion in Primates
These studies will build on previous work by systematically examining, in rhesus monkeys, the neural circuitry that mediates the regulation of long term anxiety responses.

R01MH043454 (PIs: Davidson/Kalin) 02/01/89-12/14/11
NIH/NIMH
Neural substrates of affective style and emotion regulation
The broad goal of this research is to further understand the neural circuitry that underlies individual differences in emotion regulation and to determine the nomological network of associations with these individual differences.

[redacted] 09/01/09–08/31/12 (NCE)
Defining Corticotropin-Releasing Factor (CRF) System Changes in Amygdala and Medial Temporal Cortex in Association with Depression and Suicide

R01MH081884-01A1 (PI: Kalin) 09/17/08-09/30/12 (NCE)
NIH/NIMH
Brain Mechanisms mediating Genetic Risk for Anxiety and Depression
The goal of the proposed experiments is to identify genetic factors that play a major role in the development of human anxiety and affective disorders.

secondary names, identifiers, and locations - excluded by agreement

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*: 07-01-2015 **End Date*:** 06-30-2016 **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				18,150.00	6,480.00	24,630.00
2 .	Dr.	name			PhD	Co-Investigator					22,147.00	7,906.00	30,053.00
3 .	Dr.	name				Co-Investigator					4,723.00	1,686.00	6,409.00
4 .		name				Psychologist					13,715.00	4,896.00	18,611.00
5 .		name				Statistian					4,470.00	1,596.00	6,066.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,769.00**

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	percent effort					
2	Undergraduate Students				10,400.00	624.00	11,024.00
	Secretarial/Clerical						
1	Marissa Riedel				10,885.00	3,886.00	14,771.00
1	Lab manager				18,592.00	6,637.00	25,229.00
1	Research Specialist				24,500.00	8,747.00	33,247.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**84,271.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**170,040.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2015 **End Date*:** 06-30-2016 **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)

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*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,478.00
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 . Animal Expenses	248,718.00
9 . Scans	42,500.00
10 . Subject Payments	31,000.00
Total Other Direct Costs	**329,696.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,736.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	499,736.00	264,860.00
		Total Indirect Costs	**264,860.00**

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)	**764,596.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: BUDGET_JUSTIFICATION1017091692.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*: 07-01-2016 **End Date*:** 06-30-2017 **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	base salary & percent effort				18,150.00	6,480.00	24,630.00
2 .	Dr.	name			PhD	Co-Investigator					22,147.00	7,906.00	30,053.00
3 .	Dr.	name				Co-Investigator					4,723.00	1,686.00	6,409.00
4 .		name				Psychologist					18,287.00	6,528.00	24,815.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **85,907.00**

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				10,400.00	624.00	11,024.00
	Secretarial/Clerical						
1	Marissa Riedel				10,885.00	3,886.00	14,771.00
1	Lab manager				12,395.00	4,425.00	16,820.00
1	Research Specialist				24,500.00	8,747.00	33,247.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**75,862.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**161,769.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2016 **End Date*:** 06-30-2017 **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*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,478.00
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 . Animal Costs	194,874.00
9 . Scans	82,500.00
10 . Subject Payments	47,000.00
Total Other Direct Costs	**334,852.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**496,621.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	496,621.00	263,209.00
		Total Indirect Costs	**263,209.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**759,830.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: BUDGET_JUSTIFICATION1017091692.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*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

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

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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				18,150.00	6,480.00	24,630.00
2 .	Dr.	name			PhD	Co-Investigator					55,368.00	19,766.00	75,134.00
3 .	Dr.	name				Co-Investigator					9,446.00	3,372.00	12,818.00
4 .		name				Psychologist					18,287.00	6,528.00	24,815.00
5 .		name				Statistian					4,471.00	1,596.00	6,067.00
6 .	Dr.	name			PhD	Scientist					65,000.00	23,205.00	88,205.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **231,669.00**

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	percent effort					
2	Undergraduate Students				10,400.00	624.00	11,024.00
	Secretarial/Clerical						
1	Marissa Riedel				10,885.00	3,886.00	14,771.00
1	Lab manager				12,395.00	4,425.00	16,820.00
1	Information Processing Specialist				30,000.00	10,710.00	40,710.00
1	Research Specialist				24,500.00	8,747.00	33,247.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**116,572.00**

Total Salary, Wages and Fringe Benefits (A+B) **348,241.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2017 **End Date*:** 06-30-2018 **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)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

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 3

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

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

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,612.00
2. Publication Costs	2,500.00
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	21,102.00
9 . Scans	77,500.00
10 . Subject Payments	31,000.00
Total Other Direct Costs	**142,714.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**495,955.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	495,955.00	262,856.00
		Total Indirect Costs	**262,856.00**

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)	**758,811.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: BUDGET_JUSTIFICATION1017091692.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*: 07-01-2018 **End Date*:** 06-30-2019 **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				18,150.00	6,480.00	24,630.00
2 .	Dr.	name			PhD	Co-Investigator					55,368.00	19,766.00	75,134.00
3 .	Dr.	name				Co-Investigator					23,615.00	8,431.00	32,046.00
4 .		name				Psychologist					18,287.00	6,528.00	24,815.00
5 .		name				Statistian					4,471.00	1,596.00	6,067.00
6 .	Dr.	name			PhD	Scientist					65,000.00	23,205.00	88,205.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **250,897.00**

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				10,400.00	624.00	11,024.00
	Secretarial/Clerical						
1	Information Processing Specialist				45,000.00	16,065.00	61,065.00
1	Research Specialist				24,500.00	8,747.00	33,247.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**105,336.00**

Total Salary, Wages and Fringe Benefits (A+B) **356,233.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2018 **End Date*:** 06-30-2019 **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)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

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

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

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

ORGANIZATIONAL DUNS*: 161202122

Budget Type*: ● Project ❍ Subaward/Consortium

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

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,037.00
2. Publication Costs	2,500.00
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	5,026.00
9 . Scans	72,500.00
10 . Subject Payments	36,000.00
Total Other Direct Costs	**131,063.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**492,296.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	492,296.00	260,917.00
		Total Indirect Costs	**260,917.00**

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

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**753,213.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: BUDGET_JUSTIFICATION1017091692.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*: 07-01-2019 **End Date*:** 06-30-2020 **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				18,150.00	6,480.00	24,630.00
2 .	Dr.	name			PhD	Co-Investigator					55,368.00	19,766.00	75,134.00
3 .	Dr.	name				Co-Investigator					23,615.00	8,431.00	32,046.00
4 .		name				Psychologist					13,715.00	4,896.00	18,611.00
5 .		name				Statistian					8,941.00	3,192.00	12,133.00
6 .	Dr.	name			PhD	Scientist					65,000.00	23,205.00	88,205.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **250,759.00**

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	percent effort					
2	Undergraduate Students				10,400.00	624.00	11,024.00
	Secretarial/Clerical						
1	Information Processing Specialist				45,000.00	16,065.00	61,065.00
1	Research Specialist				24,500.00	8,747.00	33,247.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**105,336.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**356,095.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2019 **End Date*:** 06-30-2020 **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)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

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*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	12,225.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	76,852.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Scans	35,000.00
9 . Subject Payment	21,000.00
Total Other Direct Costs	**147,577.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**508,672.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53	456,820.00	242,115.00
		Total Indirect Costs	**242,115.00**

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)	**750,787.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: BUDGET_JUSTIFICATION1017091692.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 funded and pending grants. R01-MH046729 "Development and Regulation of Emotion in Primates" uses viral vector techniques to directly manipulate CRH and NTF-3 expression in the Ce. R01-MH081884 "Brain Mechanisms Mediating Genetic Risk For Anxiety and Depression" uses a combination of PFC lesions, brain imaging, and molecular genetics in young monkeys to focused on molecular alterations in the Ce that relate to AT. Project 1 of the Conte Center P50-MH100031 grant, "Neural mechanisms mediating adversity's impact on the risk for developing anxiety" proposes to assess mechanisms of perturbed early rearing environment on the development of primate anxiety-related psychopathology. Distinct from the above research, the current proposal is complementary by using multimodal neuroimaging methods to study young girls with extreme anxiety as they transition to adolescence with a focus on sustained anxiety responses in the bed nucleus of the stria terminalis (BST). A parallel nonhuman primate arm of the study will test the causal role of the BST in anxious behavior using our well-validated homologue of childhood anxiety, anxious temperament (AT) in a sample of young female rhesus monkeys. Finally, we will use state-of-the-art deep RNA sequencing of neurons tissue harvested from the BST in already-collected and phenotyped female rhesus brains to characterize the molecular mechanisms underlying BST metabolism and the expression AT.

KEY PERSONNEL

Ned H. Kalin, MD: Principal Investigator (percent effort)
Dr. Kalin will serve as Principal Investigator of this project, overseeing all related studies. He has more than 26 years of experience in primate research, and has been involved in human functional imaging studies in adults with anxiety and depression for the last 18 years in addition to conducting neuroimaging studies of children with anxiety disorders for the last 4 years. Dr. Kalin will design experiments, oversee subject selection for studies of anxious girls, interpret results, prepare manuscripts and coordinate interactions with key personnel and collaborators. Salary calculation is based on the $199,700 of legislatively imposed salary cap for Year 1.

name , PhD: Co-Investigator (percent effort Year 1 and 2, percent effort Year 3-5)
name 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 human imaging data and integration of these measures with behavioral and physiological data. He has conducted studies using fMRI in humans and 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 human and non-human primates. Due to his anatomical expertise and his experience over the past year with LCM, he will lead the effort to collect neurons from the BST, with increased effort in years 3-5 to do so. Addition effort in Years 4 and 5 will also be allocated for data analysis and manuscript preparation.

name , PhD: Co-Investigator (percent effort Year 1 and 2, percent effort Year 3, percent effort perce ear 4 and 5)
name has 25 years of experience with central nervous system molecular biology and pharmacology. For the nonhuman primate studies he will oversee the RNA purification and amplification in preparation for deep RNA sequencing from banked brains and supervise the work on the extraction of RNA and DNA from whole blood from lesion sample. He will assist in phenotyping the monkeys by measuring plasma levels of cortisol, which is one of the measures that comprise the AT metric. For human imaging studies he will perform all hormone assays and DNA extraction from buccal swabs. In Years 3-5 name effort will percent effort assist with LCM, conduct analyses and contribute to interpretation and manuscript preparation related to the hormonal and neuroimaging data.

name , PhD, Psychologist, percent effort in Years 1 and 5, percent effort in Years 2-4 - name will conduct K-SADS interviews for anxious girls enrolled in the study. name has specialized in the diagnosis and treatment of childhood anxiety disorder for the last 13 years and has worked with Dr. Kalin for the last 4 years in a similar role on studies of pediatric anxiety disorders. Effort is percent effort in Years 3-5 to accommodate additional subjects continuing in longitudinal design.

name PhD, Scientist percent effort in Years 3-5 - name is a postdoctoral fellow in the Kalin lab and will join the research team in Years 3-5 after the completion of her fellowship. She will work closely with name r and Dr. Kalin on collection, analysis, and interpretation of human neuroimaging data as well as manuscript preparation. name has 6 years' experience with functional brain imaging research and has worked with Drs. Kalin and name on studies of childhood anxiety disorders for the last 2 years.

name PhD percent effort Years 1, 3 and 4; percent effort in Year 5 – name will work closely with Dr. Kalin and the research team to supervise statistical analyses of human neuroimaging data using latent growth curve models. name has more than 12 years of experience using these methods to analyze longitudinal datasets, and designed the analytic plan in the proposal. Her effort percent effort in Year 5 during final analyses of full dataset.

KEY PERSONNEL – Other Significant Contributor with percent effort efforts

name, PhD – OSC name study which followed a large cohort of individuals from before birth to adulthood and provide essential insight into child development, stress, and psychopathology. name and Dr. Kalin have worked closely together for more than 10 years, and she will lend her expertise in longitudinal studies to promote recruitment and retention for the proposed projects, as well as methods for administering and analyzing data from the Trier Social Stress Test.

name, PhD – OSC name is an Assistant Professor at Harvard University whose research focuses on drawing mechanistic links between brain development, emotional processes, and risk for psychopathology. name developed the sustained anxiety neuroimaging paradigm we will use in this proposal, which she recently used to collect a large sample of normative data from participants aged 9-22. She will serve as a consultant for data collection, analysis and interpretation on this paradigm.

name, MD – OSC name and a leading expert on neuroimaging studies of anxiety disorders. name has collaborated with the Kalin lab over the last 4 years on studies of childhood anxiety disorders. He will serve as a consultant related to clinical diagnoses, clinical assessments, and the interpretation of neuroimaging data for the proposed study.

name, PhD – OSC name and will serve as a consultant on this project for five years. He is internationally renowned for his research on the neural substrates of emotion and emotional disorders and will lend his expertise to issues related to data analyses and interpretation of results with Dr. Kalin and other key personnel.

name, PhD – OSC name, and an expert in mapping and measuring the functional and structural organization of the human brain using MRI. In this proposed research, name will serve as a consultant on structural imaging methods development and analysis.

name, PhD – OSC name is an Assistant Professor of Psychiatry and Medical Physics. 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 on resting fMRI measures of functional connectivity. name will serve as a Consultant in the proposed study.

name, PhD – OSC name is a Professor of Biomedical Engineering, Medial Physics, and Radiology at the University of Wisconsin-Madison, 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 will lend his expertise for MRI guided targeting for the lesion studies in this proposal.

OTHER PERSONNEL

name : Senior Research Specialist percent effort in Year 1, percent effort in Years 2 and 3)
name has more than 28 years of research experience in primate behavior. 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, name will assist with physiological sampling and will oversee the adherence to the strict regulations by providing documentation requirements unique to primate research. percent effort effort is allocated in Year 1 for screening animals.

name : Research Specialist percent effort in Years 1-3)
name 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.

TBN Research Specialist, percent effort
Working with name this individual will conduct and oversee online screening, assessment, and data collection for anxious girls.

TBN Information Processing Specialist, percent effort in Year 3, percent effort in Years 4 and 5. After the first wave of data collection this individual will assist in updating and optimizing MRI processing pipelines, data organization and backup, and data storage to accommodate the number of MRI scans in this proposal. This individual will increase their effort in Years 4 and 5 as longitudinal data accrue to assist in analysis pipelines to examine longitudinal changes within subjects, and optimize processing of final dataset as the study concludes.

Students – percent effort in Years 1-5: Under the guidance of full time staff students will provide assistance for human and nonhuman primate studies. For human studies, student workers will assist with data acquisition, data entry, and scheduling of study appointments For nonhuman primate studies students will assist with 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

Total Personnel Cost for Entire Period: $1,392,378

NON-PERSONNEL BUDGET
Listed non-personnel budget is for the entire period of five years. The non-personnel costs might incur in a more concentrated manner during certain grant periods.

SUPPLIES

Ancillary:

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

Total Ancillary Costs for Entire Period: $22,500

Assay for Nonhuman Primate Studies:

Costs include standards, tubes, reagents, and radioactive tracers for extraction of RNA and plasma cortisol obtained during hormonal sampling.

Tissue – from 24 monkeys in our phenotyped primate brain bank
RNA Extraction Kits

- Direct-zol RNA miniprep kits for purification of total RNA including both mRNA and small RNAs ≥ 17 nucleotides
- 24 subjects with 1 sample each (BST) = 24 samples
  1 kit = 50 samples @ $226 / kit = $226
  Total = $226 in Year 4

RNA Amplification

- NuGen Ovation RNA-Seq System V2
- 24 subjects with 1 sample each (BST) = 24 samples
  1 kit = 32 sample @ $4,800 / Kit = $4,800
  Total = $4,800 in Year 4

Blood – from 20 monkeys in lesion study
PAXgene Blood RNA tubes for collection and PAXgene Blood miRNA kits for purification of total RNA including both mRNA and small RNAs > 18 nucleotides. Costs include RNA tubes and kits.
Year 2: 18 subjects with 4 samples each = 72 samples @ $22.60/sample = $1,627
Year 3: 2 subjects with 4 samples each = 8 samples @ $22.60/sample = $180

PAXgene Blood DNA tubes for collection only
Year 2: 18 subjects @ $22.60/sample = $121
Year 3: 2 subjects @ $6.75/sample = $13

Plasma
Year 1

- Vet tech assistance for CSF draw for 18 subjects with 2 draws each = 36 draws @ $50/draw = $1,800
- CSF/CRH Assay for 18 subjects with 2 samples each for CRF assay = 36 samples @ $7/draw = $252
- Cortisol: 18 subjects with 9 samples each + 2 subjects with 5 samples each = 172 samples @ $8 = $1,376

Year 2

- Vet tech assistance for CSF draw for 20 subjects with 2 draws each = 40 draws @ $50/draw = $2,000
- CSF/CRH Assay for 20 subjects with 2 draws each = 40 samples @ $7/draw = $280
- Cortisol: 8 subjects with 7 samples each + 10 subjects with 6 samples each + 2 animals with 10 samples each = 136 samples @ $8 = $1,088

Year 3

- Vet tech assistance for CSF draw for 2 subjects with 2 draws each = 4 draws @ $50/draw = $200
- CSF/CRH Assay for 2 subjects with 2 draws each = 4 samples @ $7/draw = $28
- Cortisol: 2 subjects with 6 samples each = 12 samples @ $8 = $96

Total Assay for Nonhuman Primate Studies Costs for Entire Period: $14,087

Surgery for Nonhuman Primate Studies:

Surgical 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.

Surgical lesion of the BST

- Year 1: 9 subjects with 1 surgery each = 9 surgeries @ $12,800 / surgery = $115,200 in Year 1

- Year 2: 1 subject with 1 surgery = 1 surgeries @ $12,800 / surgery = $12,800 in Year 2

Total Surgery Costs for Entire Period: $128,000

Assay for Human Studies:

Pubertal Hormones:

Costs include standards, tubes, reagents, and radioactive tracers for measurement of salivary cortisol, testosterone, and estradiol obtained during hormonal sampling

Year 1

- Cortisol: 85 Initial MRI subjects with 3 samples each + 85 TSST subjects with 3 samples each = 510 sample @ $6.75/sample = $3,442
- Testosterone: 85 Initial MRI subjects @ $7.50/sample = $637
- Estradiol: 85 Initial MRI subjects @ $9.75/sample = $828

Year 2

- Cortisol: 85 Initial MRI subjects + 80 Assessment 2 MRI subjects with 3 samples each + 85 TSST subjects with 3 samples each = 750 samples @ $6.75/sample = $5,062
- Testosterone: 85 Initial MRI subjects + 80 Assessment 2 MRI subjects = 165 samples @ $7.50/sample = $1,237
- Estradiol: 85 Initial MRI subjects + 80 Assessment 2 MRI subjects = 165 samples @ $9.75/sample = $1,608

Year 3

- Cortisol: 80 Assessment 2 MRI subjects + 75 Assessment 3 MRI subjects with 3 samples each = 465 samples @ $6.75/sample = $3,138
- Testosterone: 80 Assessment 2 MRI subjects + 75 Assessment 3 MRI subjects = 155 samples @ $7.50/sample = $1,162
- Estradiol: 80 Assessment 2 MRI subjects + 75 Assessment 3 MRI subjects =155 samples @ $9.75/sample = $1,511

Year 4

- Cortisol: 75 Assessment 3 MRI subjects + 70 Assessment 4 MRI subjects with 3 samples each = 435 samples @ $6.75/sample = $2,936
- Testosterone: 75 Assessment 3 MRI subjects + 70 Assessment 4 MRI subjects = 145 samples @ $7.50/sample = $1,087
- Estradiol: 75 Assessment 3 MRI subjects + 70 Assessment 4 MRI subjects = 145 samples @ $9.75/sample = $1,413

Year 5

- Cortisol: 70 Assessment 4 MRI subjects with 3 samples each = 210 samples @ $6.75/sample = $1,417
- Testosterone: 70 Assessment 4 MRI subjects @ $7.50/sample = $525
- Estradiol: 70 Assessment 4 MRI subjects @ $9.75/sample = $682

DNA collection, extraction, and storage:

Costs include buccal swabs for collection, and costs for materials to extract DNA

- Year 1: 85 subjects @$2 each = $170
- Year 2: 85 subjects @$2 each = $170

Total Assay for Human Studies Costs for Entire Period: $27,025

OTHER COSTS

PET Scans:

Costs include preparation of FDG, technician time, and use of scanner.

Year 1:

- 18 subjects (9 lesion, 9 control) with 2 scans each (pre lesion, 3 months post lesion) = 36 scans (1 hr/scan) @ 475/hr = $17,100
- 36 doses @ $135 / FDG dose = $4,860

Year 2:

- 18 subjects (9 lesion, 9 control) with 1 scan each (1 year post lesion) + 2 subjects (1 lesion, 1 control) with 2 scans each (pre lesion, 3 months post lesion) = 22 scans (1 hr/scan) @ 475/hr = $10,450
- 22 doses @ $135 / FDG dose = $2,970

Year 3:

- 2 subjects with 1 scan each (1 year post lesion) = 2 scans (1 hr/scan) @ 475/hr = $950
- 2 doses @ $135 / FDG dose = $270

Total PET Scanning Costs for Entire Period: $36,600

MRI Scans Nonhuman Primate Studies:

Costs include use of scanner and technician time. MRIs are necessary for co-registration of microPET data and to individually target BST lesions, and will be performed at the same intervals as PET scans.

- Year 1: 18 subjects (9 lesion, 9 control) with 2 scans each (pre lesion, 3 months post lesion) = 36 scans (1.5 hr/scan) @ 500/hr = $27,000
- Year 2: 18 subjects (9 lesion, 9 control) with 1 scan each (1 year post lesion) + 2 subjects (1 lesion, 1 control) with 2 scans each (pre lesion, 3 months post lesion) = 22 scans (1.5 hr/scan) @ 500/hr = $16,500
- Year 3: 2 subjects with 1 scan each (1 year post lesion) = 2 scans (1.5 hr/scan) @ 500/hr = $1,500

Total Nonhuman Primate MRI Scanning Costs for Entire Period: $45,000

Pathology:

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

- Year 2: 9 subjects @ $1,200 / subject = $10,800
- Year 3: 1 subject @ $1,250 / subject = $1,200

Total Pathology Costs for Entire Period: $12,000

Animal Replacement:

9 subjects will be replaced in Year 2 and 1 subject will be replaced in Year 3.

- 10 subjects x $6,150 = $61,500

Total Animal Replacement Costs for Entire Period: $61,500

Animal Per Diems:

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

Year 1:

- 100 screening subjects assigned for 2 days with 2 screenings each = 400 days @ $13.13 / day = $5,252
- 20 animals assigned for 261 days = 5220 days @ $13.13 / day = $68,538

Year 2: 20 animals assigned for 288 days = 5760 days @ $13.13 / day = $75,628

Year 3: 2 animals assigned for 210 days = 420 days @ $13.13 / day = $5,514

Animal pre-assignment physicals and clinical blood work:

- Year 1: 18 subjects @ $130 / subject = $2,340
- Year 2: 2 subjects @ $130 / subject = $260

Total Animal Costs for Entire Period: $*157,532*

MRI Scans for Human Studies:

Costs include use of scanner and technician time and radiological review for a 60-minute scanning session at the rate of $500/hr. Girls will be enrolled in to cohorts, with 85 entering in Year 1 and 85 entering in Year 2.

- Year 1: 85 initial scans @ $500/hr = $42,500
- Year 2: 85 initial scans + 80 Assessment 2 scans = 165 scans @ $500/hr = $82,500
- Year 3: 80 Assessment 2 scans + 75 Assessment 3 scans = 155 scans @ $500/hr = $77,500
- Year 4: 75 Assessment 3 scans + 70 Assessment 4 scans = 145 scans @ $500/hr = $72,500
- Year 5: 70 Assessment 4 scans @ $500/hr = $35,000

Total MRI for Human Studies Scanning Costs for Entire Period: $310,000

Subject Payment:

Subjects will be paid $20 for online screening, $50 for KSADS visit, $150 for MRI and EMG sessions, and $50 for TSST visit. Subject completing all study visits for which they qualify will earn a $100 study completion bonus.

Year 1:

- Online screening of 450 subjects @ $20 = $9,000
- Initial KSADS of 100 subjects @ $50 = $5,000
- Initial MRI and EMG for 85 subjects @ $150 = $12,750
- TSST for 85 subjects @ $50 = $4250

Year 2:

- Online screening of 450 subjects @ $20 = $9,000
- Initial KSADS of 100 subjects + Assessment 2 KSADS of 80 subjects = 180 KSADS @ $50 = $9,000
- Initial MRI and EMG for 85 subjects + Assessment 2 MRI and EMG of 80 subjects = 165 MRI and EMG sessions @ $150 = $24,750
- TSST for 85 subjects @ $50 = $4250

Year 3:

- Assessment 2 KSADS of 80 subjects + Assessment 3 KSADS of 75 subjects = 155 KSADS @ $50 = $7,750
- Assessment 2 MRI and EMG for 80 subjects + Assessment 3 MRI and EMG of 75 subjects = 155 MRI and EMG sessions @ $150 = $23,250

Year 4:

- Assessment 3 KSADS of 75 subjects + Assessment 4 KSADS of 70 subjects = 145 KSADS @ $50 = $7,250

- Assessment 3 MRI and EMG for 75 subjects + Assessment 4 MRI and EMG of 70 subjects = 145 MRI and EMG sessions @ $150 = $21,750
- Study completion bonus for 70 subjects @ $100 = $7,000

Year 5:

- Assessment 4 KSADS of 70 subjects @ $50 = $3,500
- Assessment 4 MRI and EMG of 70 subjects = 70 MRI and EMG sessions @ $150 = $10,500
- Study completion bonus for 70 subjects @ $100 = $7,000

Total Subject Payment Costs for Entire Period: $166,000

Data Storage Costs

Data storage costs include regular backup and data security. The amount of storage needed increases as the data set increases in size.

- Year 1: 1 TB @ $2400/TB = $2,400
- Year 2: 1 TB @ $2400/TB = $2,400
- Year 3: 2 TB @ $2400/TB = $4,800
- Year 4: 4 TB @ $2400/TB = $9,600
- Year 5: 4 TB @ $2400/TB = $9,600

Total Data Storage Costs for Entire Period: $28,800

Travel:
Travel cost is requested for the PI and research staff to present findings as neuroscientific and psychiatric conferences
$5,000 / year in Years 3 - 5

Total Travel Costs for Entire Period: $15,000

Total UW-Madison Direct Cost for Entire Period: $2,416,428

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**905,001.00**
Section B, Other Personnel		**487,377.00**
Total Number Other Personnel	24	
Total Salary, Wages and Fringe Benefits (A+B)		**1,392,378.00**
Section C, Equipment		
Section D, Travel		**15,000.00**
1. Domestic	15,000.00	
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		**1,085,902.00**
1. Materials and Supplies	55,830.00	
2. Publication Costs	7,500.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	76,852.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	504,720.00	
9. Other 2	296,000.00	
10. Other 3	145,000.00	
Section G, Direct Costs (A thru F)		**2,493,280.00**
Section H, Indirect Costs		**1,293,957.00**
Section I, Total Direct and Indirect Costs (G + H)		**3,787,237.00**
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*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		name	[redacted]	[redacted]		PD/PI	base salary & percent effort				8,561.00	2,661.00	11,222.00
2 .		name		[redacted]		Co-Investigator					3,413.00	1,061.00	4,474.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **15,696.00**

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	Lab Technician	percent effort			5,101.00	1,148.00	6,249.00
1	Bioinformaticist/Programer				7,357.00	2,288.00	9,645.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**15,894.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**31,590.00**

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

Funding Opportunity Number: PA-13-302 . Received Date: 2014-10-03T16:56:13.000-04:00

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*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 1**

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

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

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**	

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

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

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

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	11,580.00
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 . Service Contracts	1,500.00
Total Other Direct Costs	**13,080.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**47,170.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	65	45,665.00	29,682.00
		Total Indirect Costs	**29,682.00**

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)	**76,852.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: [redacted]_Budget_Justification1017091805.pdf
(Only attach one file.)

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

USC BUDGET JUSTIFICATION

According to a DHHS rate agreement dated 6/23/2014, the USC fringe benefit rate is 31.1% and the F&A rate is 65%. Salaries are capped at the anticipated NIH Executive Level II cap where relevant.

PERSONNEL

[redacted] **effort in Year 5).** [redacted] He is both a board-certified psychiatrist and a well-established human geneticist with years of experience in largescale 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. [redacted] 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 Dr. [redacted] and the laboratory staff to oversee their work, and will have monthly conference calls and biannual meetings with the UW site to discuss progress.

[redacted] **PhD, Co-Investigator (**[redacted] **effort in Year 5).** Dr. Deng is an Assistant Professor of Research at the Department of Psychiatry and the Behavioral Sciences at the Keck School of Medicine, and an accomplished human geneticist. Along with Dr. [redacted], he discovered the gene for primary pulmonary hypertension (PPH). The discovery of this gene, which encodes the Bone Morphogenetic Protein Receptor II (*BMPR2*) has opened up a substantial area of research into the importance of the BMP pathway to the maintenance of vascular integrity in humans. Dr. [redacted] will be primarily responsible for the RNA sequencing of the project and will direct [redacted] the laboratory technician.

[redacted]**, PhD, Research Associate (**[redacted] **effort in Year 5).** [redacted] will maintain the Genome Analyzer “pipeline” software, manage our various “pools” of data storage, install and test new programs on the USC HPCC cluster, install and maintain additional local computational nodes running VMware-ESXi to virtualize all local project servers, install and maintain a project Wiki, perform the RNA-Seq analysis and maintain the Progeny LIMS system which will be used to manage sample flow.

[redacted] **Laboratory Technician (**[redacted] **effort in Year 5).** Mr. [redacted] is an experienced laboratory technician. He is facile with all aspects of the laboratory portion of the RNA-Seq workflow, including QC of RNA samples, library construction, library QC, cluster formation and sequencing on the HiSeq2500’s.

SUPPLIES

RNA Sequencing Library Construction. $1,680 is requested in Year 5 to purchase Illumina TruSeq RNA Sample Prep Kits with sample indexing sufficient to build libraries.

RNA Sequencing Reagents. $8,400 is requested in Year 5 for reagents to perform RNA sequencing on an Illumina HiSeq2500 sequencer.

TRAVEL

Scientific Meetings. $2,500 is requested in Year 5 for trips to the University of Wisconsin to attend project meetings.

OTHER EXPENSES

Service Contracts. $1,500 is requested in Year 5 for this project's portion of the service contracts necessary to maintain an Illumina HiSeq2500, cBOT cluster station, laboratory freezers, etc.

EQUIPMENT

Supercomputer Nodes and Disk Drives. $1,500 is requested in Year 5 for compute nodes and RAID Array Disks to be installed and maintained (at no cost) at the USC High Performance Computing & Communications (HPCC) cluster. name [redacted] is on the Faculty Advisor Board to the HPCC. Nodes in the USC HPCC are "retired" after 3 years and will need to be replaced. It is also possible we will need to purchase additional disk storage space (local or at our HPCC) for this project.

These costs are included in equipment (excluded from MTDC F&A calculations) because this purchase will be included as part of a larger purchase exceeding $5,000.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**15,696.00**
Section B, Other Personnel		**15,894.00**
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		**31,590.00**
Section C, Equipment		
Section D, Travel		**2,500.00**
1. Domestic	2,500.00	
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		**13,080.00**
1. Materials and Supplies	11,580.00	
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	1,500.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**47,170.00**
Section H, Indirect Costs		**29,682.00**
Section I, Total Direct and Indirect Costs (G + H)		**76,852.00**
Section J, Fee		

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	Risk_SpecificAims_final1017091715.pdf
3. Research Strategy*	Risk_ResearchPlan_FINALFRIDAY1017091915.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Risk_Protection_Human_Subjects_final1017091706.pdf
6. Inclusion of Women and Minorities	Risk_InclusionWomenMinorities_final1017091721.pdf
7. Inclusion of Children	Risk_Inclusion_of_Children_final1017091804.pdf
Other Research Plan Sections	
8. Vertebrate Animals	Risk_Vertebrate_animals_final1017091709.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	name _Face_Page1017091726.pdf
12. Letters of Support	LOS21017091918.pdf
13. Resource Sharing Plan(s)	Risk_ResourceSharing_final1017091714.pdf
Appendix (if applicable)	
14. Appendix	

Specific Aims: Persistent and high levels of sustained anxiety during childhood are among the best predictors of the later development of anxiety and depressive disorders during adolescence. This is particularly relevant to females, as after puberty girls are twice as likely to develop these disorders. The goal of this proposal is to understand the biological mechanisms of sustained anxiety in highly anxious girls, how it changes over time, and how it can transform into psychopathology. From our longitudinal community sample, we followed highly anxious 11-year-old girls through age 15. While some girls naturally overcame their anxiety, ~40% stayed highly anxious or worsened. Additional behavioral, physiological, and neuroimaging data from our laboratory studying these children, as well as studies of a model of dispositional anxiety in young nonhuman primates, underscores that highly anxious primates are extremely sensitive to uncertain threats. Although sustained anxiety responses can be adaptive, many patients with stress-related psychopathology experience extreme levels of maladaptive sustained anxiety when the likelihood, quality, and/or timing of a negative event is uncertain. Maladaptive, uncertainty-related anxiety is a trans-diagnostic feature that underlies internalizing symptoms such as pathological worry, anxious rumination, difficulty concentrating, and anticipatory anxiety. While the intensity of these symptoms varies across individuals and diagnoses, they commonly occur in patients with anxiety and depression. Importantly, approximately half of the young girls from our community sample with high dispositional anxiety developed anxiety and/or depressive disorders.

Here, we will use a translational approach to understand the neurobiology of sustained anxiety by studying highly anxious young girls and young anxious female monkeys as they transition into adolescence. We will focus on the bed nucleus of the stria terminalis (BST) because it is thought to be involved in sustained anxiety and the maintenance of prolonged threat preparedness. Similar paradigms will be used in humans and monkeys to: 1) characterize developmental trajectories of brain function in highly anxious young human females; 2) examine the relevance of altered BST function in relation to the onset of anxiety and depressive disorders; 3) test the causal role of the BST in anxiety as young anxious female monkeys mature into adolescence; and 4) define BST molecular alterations that are linked to altered BST function and sustained anxiety. Importantly, the BST is part of a larger network involved in the adaptive and maladaptive expression of anxiety, and the function of this network will also be examined in relation to BST, psychopathology, and development.

Aim 1: Anxiety Trajectories. 170 highly anxious 10 – 11 year old girls will be followed for 3 years with yearly multimodal neuroimaging, clinical, and behavioral assessments. We will use a neuroimaging paradigm that is ideally suited to elicit BST activation and sustained anxiety by exposure to prolonged threat. We predict that the trajectory of BST activation will differ between individuals who maintain high levels of anxiety as they transition to adolescence as compared to those whose anxiety levels decrease as they mature.

Aim 2: Development of Psychopathology. In the girls that maintain their high levels of anxiety into adolescence, we will investigate the neural mechanisms that predict and reflect the development of *bona fide* psychopathology. We hypothesize that elevated BST activation specifically induced by uncertainty will be a trans-diagnostic indicator for the development of anxiety and depressive disorders in dispositionally anxious girls.

Aim 3: Causal Role of the BST. In preadolescent anxious female monkeys, we will selectively lesion neurons in the BST to test the hypotheses that the BST mediates ethologically relevant sustained anxiety that is associated with uncertainty. Mechanistically implicating the BST in extreme dispositional primate anxiety is crucial to clarify the interpretation of data from human imaging studies focused on internalizing symptoms and sustained anxiety. This will be particularly relevant to understanding the extent to which novel pharmacologic and psychological treatments should be aimed at altering BST in dispositionally anxious girls.

Aim 4: Molecular Alterations in BST Neurons. We will investigate transcriptome-wide gene expression in BST neurons to understand the molecular processes associated with BST activation and dispositional anxiety. In brains already collected from 24 phenotyped peri-adolescent female monkeys, BST neurons will be harvested using laser capture microdissection (LCM), and gene expression will be characterized with RNASeq. We predict that a deficit in the expression of specific neuroplasticity-related genes will underlie elevated BST metabolism and at-risk early-life dispositional anxiety.

The proposed studies focused on highly anxious girls as they transition to adolescence, along with the mechanistic primate studies, have the potential to provide new insights into the neurobiology of the emergence of adolescent of stress-related psychopathology.

a) Significance:

Adaptive and maladaptive sustained anxiety responses in relation to the development of anxiety and depressive disorders. The ability to anticipate threatening situations and its accompanying anxiety is critical for survival and well-being. This anticipation is characterized by sustained anxiety, which differs from acute responses to threat by allowing individuals to prepare for and effectively respond to threat. This prolonged state of vigilance and arousal is associated with the experience of anticipatory anxiety, which is heightened by the degree of uncertainty about future events [1]. Maladaptive, uncertainty-related anxiety is a trans-diagnostic feature that underlies internalizing symptoms such as pathological worry, anxious rumination, difficulty concentrating, and anticipatory anxiety. Anxiety and affective disorders are characterized by sustained anxiety and negative affect, which manifests as excessive, persistent and pervasive worries and fears. The influences of sustained anxiety during childhood and adolescence can lead to significant disability, including school avoidance, social isolation, and erosion of self-esteem, all of which are common in patients with anxiety and/or depressive disorders. In this proposal, using a translational approach we aim to gain clinical, mechanistic, and molecular insights into sustained anxiety by performing complementary studies in human and nonhuman primates. Sustained threat preparedness is an ideal construct because it is dimensional, and when excessive confers risk for anxiety and depressive disorders [2-5]. Importantly, sustained anxiety can also be modeled in nonhuman primates, providing critical translational opportunities. Anxious young females are at a particularly increased risk for developing anxiety and depressive disorders as they transition to adolescence. Persistent and high levels of sustained anxiety 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 highly anxious young females because with the transition to adolescence (age 12-14) females are twice as likely have anxiety and/or depressive disorders [9-13]. While anxiety and depressive disorders are separable they share many clinical features. Here, we focus on an important nexus between these disorders, that is, sustained anxiety and its resultant symptoms. Emerging work suggests that pubertal stage, as opposed to chronological age, is the most important factor in the elevated rate of anxiety and depression in females [10, 14, 15]. Rising levels of pubertal hormones, significant social stressors, the transition to middle school, and shifts in social relevance from parents to peers can create the perfect storm for the emergence of anxiety and depressive disorders. From a neural perspective, it has been suggested that the increased rate of anxiety and affective disorders during adolescence coincides with an “imbalance in function” between the emotion processing capacity of the limbic system with the relatively less-mature prefrontal regions that are crucial for emotion regulation [16]. We believe that this is particularly relevant to the maturation of neural systems underlying adaptive and maladaptive sustained anxiety responses. Furthermore, we emphasize the importance of heightened sensitivity to uncertain situations as being a critical component linking high levels of anxiety to the development of stress-related psychopathology. Longitudinal data focusing on the neural circuits underlying sustained anxiety collected during the critical transition to adolescence will allow us to examine the developmental trajectories of highly anxious girls. These data will inform outcomes associated with risk, resilience, and the development of psychopathology and have the potential to establish biomarkers that identify individuals that will continue to suffer as they mature versus those that get better on their own. Developing interventions for at risk girls is a high priority and will benefit from understanding alterations in the neural systems that underlie maladaptive sustained anxiety during the transition from risk to psychopathology. Neurodevelopment across the transition from late childhood to adolescence. Neurodevelopmental time course issues are important for understanding the emergence of psychopathology in young girls as they transition to adolescence. In this proposal we will longitudinally follow highly anxious girls for three years, beginning at age 10-11 and continuing until age 13-14. Studying children longitudinally across the transition from late childhood to early adolescence allows for the examination of neurodevelopmental changes associated with maturation that underlie sustained anxiety and responses to uncertainty. Understanding the impact of these developmental influences on the maturation of the bed nucleus of the stria terminalis (BST), a limbic region implicated in prolonged anxiety responses will be a focus of this proposal. Changing hormone levels and pubertal stage are important for understanding developmental influences on brain structure and function (e.g. [17-24]). Normal brain development is characterized by early stages of synaptogenesis and axonal growth, followed by more protracted periods of synaptic pruning and myelination. This is particularly evident in prefrontal cortex (PFC) where development of white matter tracts continues from childhood into early adulthood [25-28]. Throughout the brain, studies of gray and white matter development demonstrate that the transition from childhood to adolescence is a period of great change in cortical structure and connectivity [29-34]. Notably, many limbic regions, including the BST, continue to develop through adolescence [21, 23, 35]. Moreover, microstructure within the white-matter tracts connecting limbic structures to regulatory frontal regions continues to develop into early adulthood [36, 37]. Functional imaging data generally support the structural findings such that late childhood to early adolescence is a critical time with respect to the development of corticolimbic circuits [38-40]. Importantly, there are large individual and gender differences in these developmental trajectories [21, 23, 35]. It is intriguing to note that the increased prevalence of anxiety and depressive disorders in females that occurs with the transition to adolescence is accompanied by the emergence of sexual dimorphism in BST volume [35, 41] and BST-relevant physiological responses reflecting sustained anxiety and uncertainty [42].

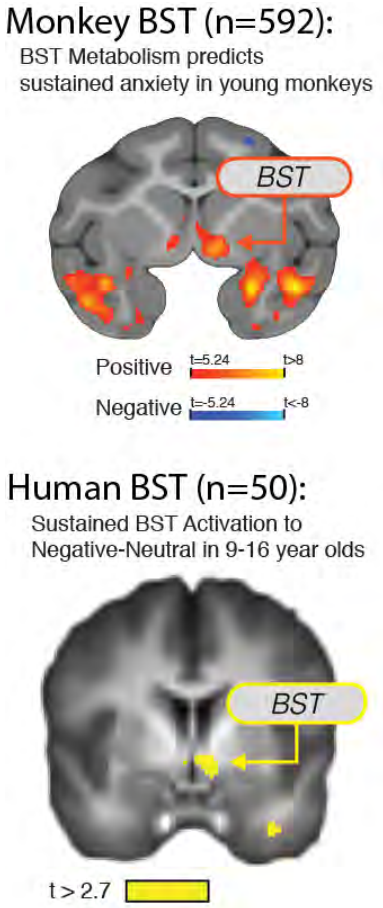

Figure 1. Cross-species BST activation. Top: Sustained anxiety-related BST activation in our monkey FDG-PET NEC paradigm. Bottom: Similar sustained BST activation in human children during the fMRI countdown task. ([redacted] unpublished data)

The neural substrates underlying sustained anxiety responses. While a multiregional network including the amygdala [43, 44] has been implicated in the generation of threat responses, behavioral and brain studies across species point to the BST as playing a central role in the generation and maintenance of sustained anxiety that, when excessive, is a central feature of anxiety and depressive disorders [45]. Our work in nearly 600 young nonhuman primates has demonstrated that BST metabolism is associated with sustained anxiety responses during exposure to a potential threat for 30 minutes [46-50] (Figure 1, top). In our nonhuman primate work, we developed a valid and highly reliable model to elicit sustained anxiety responses. Specifically, the no-eye-contact (NEC) condition of the human intruder paradigm exposes animals to an uncertain, potentially threatening predator. This condition, in which a human intruder presents their profile to the monkey to intentionally avoid eye contact, elicits robust sustained anxiety responses that are clearly differentiated from immediate fear responding induced by direct threat. Similar to our monkey studies, recent functional neuroimaging work in healthy humans demonstrates sustained BST activation during conditions that elicit sustained anxiety. For example, increasing BST activation occurs when participants observe an aversive stimulus approaching them in space or time [51-53], as well as during prolonged anticipation of a negative stimulus (e.g. negative images [54, 55] or shock [56]). Preliminary data from our collaborator [redacted] has recently extended these findings to children. For example, when 9-16 year olds view prolonged blocks of negative images they demonstrate BST activation similar to that observed in adults and non-human primates (Figure 1, bottom). While few studies of patients with anxiety disorders have focused on the BST, anxiety disorder patients exhibit elevated BST activation when exposed to conditions that provoke sustained anxiety [57-59].

The BST interacts with many other brain regions implicated in anxiety and depressive disorders, and likely serves as a substrate for shared symptoms across these disorders. As part of the “extended amygdala” [60-64], the BST and the central nucleus region of the amygdala (Ce) share strong functional and structural connections [65, 66], are composed of similar cell types [61, 62, 67], and have many similar efferent targets including the brainstem and hypothalamus which are central to mounting a fear response [68-71]. Additional anxiety-related brain areas that send afferent projections to the BST include regions of the orbitofrontal cortex (OFC) and insula [72-76]. The broader anxiety-related network is thought to work in concert to produce the behavioral and physiological responses that occur with sustained anxiety. Translational work across primate species: an opportunity to identify mechanisms relevant to sustained anxiety and the risk to develop anxiety and depressive disorders. There is no question that translational neurobiological research has promise for understanding the pathogenesis and etiology of psychiatric disorders. However, translation between animal models and humans frequently has conceptual and practical limitations in the ability to employ comparable paradigms, behavioral assessments, and outcome measures across species. In this proposal, we have the unique opportunity to utilize a well-validated non-human primate model of early-life sustained anxiety alongside studies in highly anxious girls. We will use parallel tasks in young anxious female human and nonhuman primates to assess neural activation during periods of sustained anxiety. Young anxious female monkeys will undergo the NEC paradigm, which induces a period of sustained and uncertain threat in the form of a human intruder. Complementary studies in highly anxious young girls will allow us to characterize and parse the acute and sustained responses to negative stimuli and to understand the influences of uncertainty. Studies in young girls will link feelings and clinical symptoms to brain function focusing on the role of the BST and its associated neural network. Studies in nonhuman primates will mechanistically test the role of the BST in sustained and dispositional anxiety and will identify its molecular substrates. Moreover, in both species we will collect comparable resting fMRI, DWI, and morphometric data. This integrated translational approach will allow us to capitalize on the unique strengths of human and nonhuman primate studies.

b) Innovation:

The BST is a highly relevant substrate for sustained anxiety and, potentially, for stress-related psychopathology. Much of the work implicating the BST in sustained anxiety has been performed in rodents, but unfortunately primate research has lagged behind (Figure 3). By making the BST and sustained anxiety the focus of this proposal, we are well poised to gain critical insights into the nature of dispositional anxiety and the development of anxiety and depressive disorders. Our translational approach bridges between basic

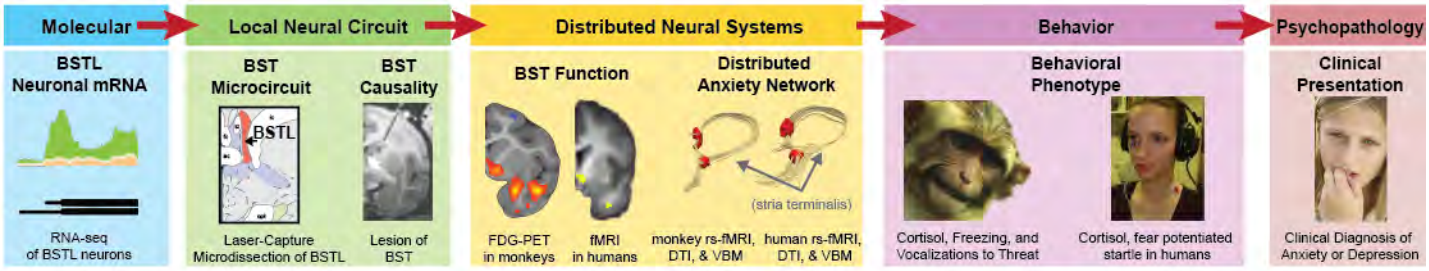

Figure 2: Integrative framework for studying anxiety and the BST

neuroscience and clinical psychopathology. Combining studies in highly anxious girls as they transition to adolescence with mechanistic studies using our non-human primate model provides the opportunity to test hypotheses about the clinical relevance and causal role of BST function. Additionally, examining the molecular composition of BST neurons provides an invaluable opportunity to identify novel and unpredicted molecular mechanisms that contribute to the at-risk child phenotype. The unique combinations of modalities and methods within this proposal are highly innovative in their combination and translational potential, as detailed below.

1) Similar neural measures across species for translation and reverse translation. We will examine the neural correlates of sustained anxiety in relation to BST function in young female primates, both human, using our "countdown task" (Figure 5) and non-human, using our well-validated rhesus NEC-imaging paradigm. Moreover, complementary imaging in both species will be performed to assess functional and structural connectivity. Using complementary imaging paradigms and methods to examine the neural responses to sustained and uncertain threat lays the foundation for our cross-disciplinary aims. **2) Extending the role of BST in dispositional anxiety and anxiety disorders.** Following highly anxious girls for three years as they transition to adolescence with prospective longitudinal brain imaging will allow us to examine the aspects of BST dysregulation that precede/reflect trajectories of sustained anxiety and/or psychopathology. This longitudinal approach is critical for understanding why some highly anxious girls naturally overcome their anxieties, while others develop anxiety and depressive disorders. Using our dimensional RDoC-like approach, we will gain insight into how the BST relates to the trans-diagnostic construct of sustained anxiety in highly anxious young girls, and how it influences the likelihood that they will manifest clinically significant anxiety and depressive symptoms. **3) Identifying the causal contribution of BST to dispositional anxiety in a primate.** The proposed experiment will be the first primate study to examine the effects of BST lesions on anxiety. Moreover, by combining this causal manipulation with functional and structural brain imaging, we will be able to examine the effects of BST lesions on the distributed neural systems that underlie sustained anxiety. This mechanistic experiment is focused on the same BST region studied in our human experiments, and will provide critical information for interpreting the correlational findings from Aims 1 & 2. **4) Exploring the molecular substrates of BST function.** Laser capture microdissection (LCM) of BST neurons for RNAseq, combined with cutting-edge bioinformatics tools and multimodal imaging affords an important opportunity to understand *how* the molecular composition of BST neurons drives dysregulated BST function and sustained anxiety.

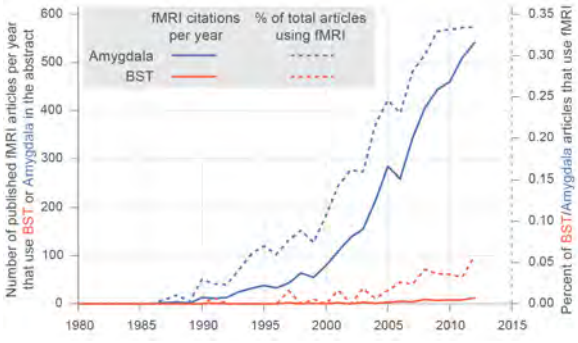

Figure 3: Automated Pubmed searches reveal < 50 fMRI articles published/year about the BST (solid-red), which is far fewer than those about the amygdala (solid-blue). Importantly, the low proportion of BST-focused fMRI articles relative to all BST-focused articles (dashed-red) reveals the need for additional BST-focused neuroimaging studies.

This proposal is designed such that each aim will provide unique insights into the nature of BST-sustained anxiety relationships by answering the questions: "*How does BST function relate to sustained anxiety over development?", "How does BST function relate to the onset of psychopathology?*", *"Is BST function causally related to sustained anxiety?", and "What molecules contribute to variation in BST function?"*. Together, these aims provide a programmatic and translational framework for linking molecular function in a critical brain substrate for sustained anxiety to dispositional anxiety and stress-related psychopathology -- all in anxious young females. The assembled research team is uniquely suited to accomplish the aims of this grant, as we have expertise in longitudinal studies ([name redacted] & Kalin), childhood brain-imaging [name redacted] Kalin), non-human primate anxiety [name redacted] & Kalin), non-human primate brain imaging ([name redacted] & Kalin), MRI-guided lesion methods ([name redacted] & Kalin), LCM of primate tissue ([name redacted] & Kalin), and RNA sequencing ([name redacted] & Kalin).

(c) Approach: Aim 1: Anxiety Trajectories. Measure anxiety trajectories to characterize neural circuit changes associated with stably high or worsening anxiety as anxious girls transition from late

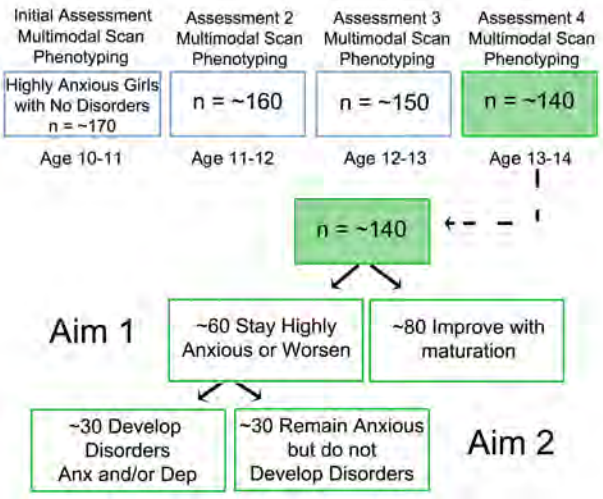

Figure 4: Study design for anxious girls.

childhood to adolescence. Our experience recruiting children for studies of childhood anxiety disorders, as well as our experience with [name redacted] in her longitudinal study (the Wisconsin Families and Work, or WISFAM study, e.g. [7, 77]), has demonstrated the availability of a large sample of preadolescent girls that are highly anxious but do not meet diagnostic criteria for anxiety or depression. Based on our preliminary analyses of the WISFAM data, enrolling 150-200 highly anxious girls will provide a sufficient sample size to test our hypotheses. 10-11 year old girls and their parents will complete online screening at two time points, ~1 months apart, to identify a sample of 200 stably high anxious girls (>10 on the Parent Screen for Child anxiety Related Disorders SCARED [78]) whose parents report that their daughter does not have a history of psychiatric illness. These 200 girls will complete an initial diagnostic interview (Kiddie Schedule for Affective Disorders and Schizophrenia, K-SADS [79]). Based on [name redacted] data we estimate that ~30 girls (15%) will be excluded for meeting criteria for a DSM-V diagnosis and will be directed to treatment. As shown in Figure 4, the remaining ~170, highly anxious, disorder-free girls will be enrolled in the study, and over the next 3 years will complete yearly assessments using multimodal neuroimaging, including our BST-focused sustained anxiety "countdown task" (Figure 5), and anxiety phenotyping using behavioral and physiological measures of sustained anxiety responses (details below). Based on the WISFAM data and our own pediatric neuroimaging studies, we estimate that about 10 girls will be lost to attrition each year, for a final sample of ~140 girls. We used the WISFAM data to estimate the proportion of girls who will maintain or have worsening levels of anxiety and will develop anxiety and/or depressive disorders. Specifically, we selected girls rated by their mothers as being highly anxious at age 11 (> 1 SD above mean) with no psychopathology. Across the transition to adolescence between ages 11-15, ~40% of these girls had high or worsening levels of anxiety whereas the remaining ~60% naturally improved with maturation. Critical for Aim 2, of those 40% who maintained stable levels of high anxiety, approximately half developed anxiety and/or depression by age 15. Latent growth curve analyses will be used to model developmental trajectories of brain function, brain structure, clinical symptoms, and anxiety-related physiological responses. [name redacted] will be instrumental in ensuring recruitment and retention given her experience with the WISFAM study, which followed a large cohort of participants from birth to 18 years old with behavioral, physiological, and neuroimaging measures (e.g. [7, 77]). Over the last 5 years, we have worked closely with [name redacted], an established expert in child psychiatry and anxiety research, such that he has been reviewing all of the K-SADs diagnoses collected in our pediatric anxiety study. He will work in conjunction with Dr. [name redacted] our clinical child psychologist and expert K-SADs rater. Trajectory analyses will be directed by [name redacted] an expert in latent growth curve methods for modeling change, individual differences, and human development using longitudinal data [80-82]. Countdown task to elicit BST activation during sustained threat and uncertainty. Anxious girls will be evaluated with a neuroimaging paradigm designed to elicit sustained anxiety-related BST activation by inducing anticipatory anxiety and negative affect under conditions of uncertainty, which are characteristic of anxiety and affective disorders. This fMRI "countdown task" uses a 2 by 2 experimental design to present participants with blocks of images that vary in their valence (negative, neutral) and predictability (certain, uncertain) (Figure 5). Within each 2-minute block of negative or neutral images, participants see a series of clock faces that are interleaved with 15 child-appropriate pictures from the International Affective Picture System (IAPS) database [83]. In the certain picture timing condition, the clocks accurately count down to picture presentation (e.g. 4,3,2,1 as shown in Figure 5A). In contrast, during the uncertain timing condition the clocks show random times that have no bearing on the timing of picture presentation (e.g. 3,1,4,6 as shown in Figure 5B). Crucially, this task allows for the dissociation of acute responses to negative pictures from sustained responding throughout negative blocks, both of which can be modulated by the predictability of picture presentation timing (Figure 5C). We are particularly interested in the sustained neural responses occurring during negative blocks as these responses parallel the persistent worries and fears that plague individuals with high levels of anxiety. Using this task in healthy adults, our collaborator Dr. [name redacted] has demonstrated a dissociation between acute

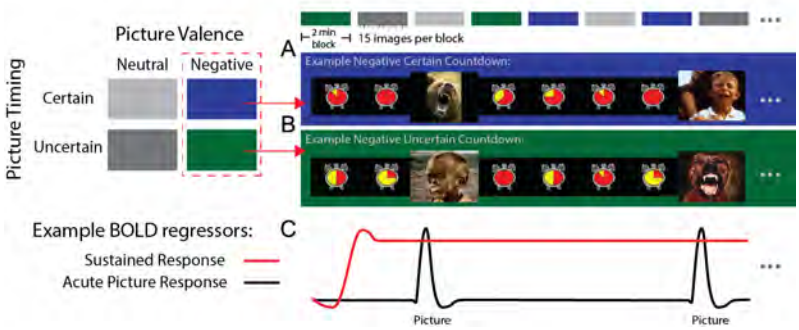

Figure 5: Example of countdown task conditions & schematic fMRI model.

and sustained responses to threat, observing acute responses to negative versus neutral images in the amygdala and sustained responses during negative versus neutral blocks in the BST [55]. More recently, the [redacted] group has extended this task to the age range we will study in this proposal, finding a similar dissociation, with robust sustained BST responses to negative versus neutral blocks in healthy, 9 – 16 year old children ([redacted] unpublished data, Figure 1). Relevant for Aim 2, greater BST activation is also seen for sustained responses during blocks with uncertain picture timing, relative to blocks with certain picture timing [55]. **We hypothesize that changes in BST activation during conditions of sustained threat (negative versus neutral picture blocks) will differentiate anxiety trajectories. Specifically, girls that maintain high anxiety or have worsening anxiety as they transition to adolescence will have stable or increasing BST responses to sustained threat. In contrast, girls whose anxiety improves with maturation will have decreasing BST responses over time. Given the central role of sustained threat response to stable high anxiety, we hypothesize that anxiety trajectories will be better predicted by sustained threat responses within the BST than by acute amygdala activation to negative pictures.** Assessing neural circuitry related to adaptive and maladaptive emotion regulation using multimodal neuroimaging including deformation-based morphometry (DBM), diffusion weighted imaging (DWI), and resting fMRI connectivity. Although our primary hypotheses are focused on the role of sustained BST activation, we will collect whole-brain functional MRI during the countdown task, as well as other complementary measures of brain structure and function. The BST receives afferent projections from varied brain areas, including the anxiety-related amygdalar, hippocampal, insular, and prefrontal regions [72-76]. Moreover, the BST's downstream effectors include hypothalamic and brainstem regions that mediate the physiological and behavioral responses to threat [68-71]. The benefit of using whole-brain, multimodal neuroimaging to study the maintenance of high levels of anxiety across development is two-fold: one, it allows for the characterization of non-BST regions that contribute to sustained anxiety responses, and two, it facilitates the understanding of the BST's role within this larger, anxiety-related network. In addition to the countdown task, we will collect structural, DWI, and resting fMRI scans to model trajectories of brain structure and BST structural and functional connectivity as anxious girls transition to adolescence. Using these techniques, our lab has identified disrupted structural and functional PFC-amygdala connectivity in preadolescent children with anxiety disorders (Figure 6; Tromp et al in prep, [84]). Consistent with delayed maturation of frontal regions for emotion regulation, **we hypothesize that OFC-BST connectivity will underlie maturational changes in BST function.** Physiological and hormonal measures related to development and sustained anxiety responses. Because we are focused on girls as they transition from late childhood to early adolescence it is crucial to evaluate the effects of changing levels of pubertal hormones and sexual maturation on brain, behavior, and sustained anxiety. Consistent with well-established protocols in Dr. Kalin's laboratory [85, 86], hormonal measurements of salivary levels of sex steroids (estradiol, testosterone) and cortisol will be performed at each assessment (details in Methods below). Physical development will be quantified using the Tanner scale [87]. The relationship between trajectories of hormonal and physical development will be compared with trajectories of brain structure, brain function, anxiety symptoms, and physiological anxiety-related responses. In addition to our BST-focused fMRI paradigm, we will measure a broad range of peripheral sustained anxiety-related responses outside the scanner using the Trier Social Stressor Test (TSST) and a sustained threat, eye-blink startle potentiation paradigm. These measures reflect RDoC dimensions within the construct of sustained threat (increased arousal and vigilance), are characteristic of stress-related psychopathology and will be used to link imaging findings to individual differences in anxiety trajectories. The TSST is a naturalistic induction of sustained psychosocial stress, consisting of performing a speech and mental arithmetic in front of panel of stoic judges that reliably elicit increases in cortisol and allows for behavioral coding of anxiety behaviors such as reduced speech output (Figure 7). We have collected TSST data on >100 adolescents and the procedures are well-operationalized within our group [88]. Because the TSST requires debriefing the participant, it will only be performed only at the initial assessment. **We predict that sustained anxiety responses during the TSST (less speech, increased reactive cortisol) will be predicted by sustained BST response during the fMRI countdown task.** In addition, we will longitudinally assess girls with a startle potentiation paradigm pioneered by Davis and Grillon that quantifies potentiation of the eye-blink startle response to acute vs. sustained anticipation of threat [45, 89]. As is

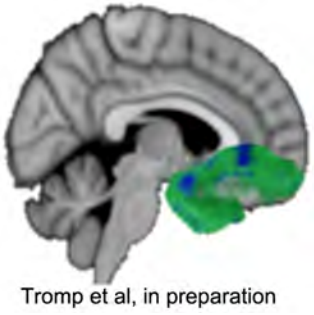

Figure 6: In blue, reduced fractional anisotropy in regions of the uncinate fasciculus in preadolescent children with anxiety disorders.

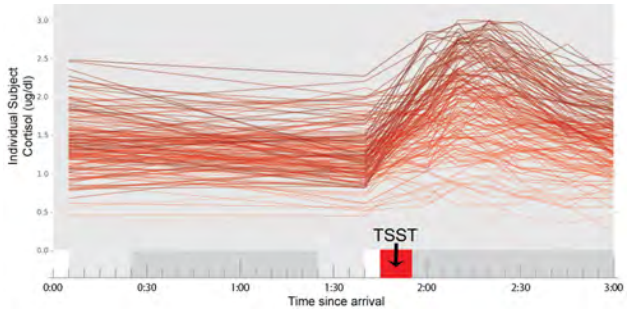

Figure 7: Cortisol levels during the TSST. Lines represent individual adolescent subjects. While there is generally a large increase in cortisol during the TSST, there is a great deal of individual variability.

commonly performed in Dr. [name] laboratory, each year we will assess the influences of acute vs. sustained threat on the startle response. To elicit startle responses we will use an effective and child appropriate stimulus, a mildly aversive blast of air to the neck [42]. **We hypothesize that individual differences in the trajectory of sustained BST activation to negative pictures assessed in the scanner will predict the magnitude of startle potentiation during sustained threat anticipation assessed outside the scanner.**

Aim 2: Development of Psychopathology. Investigating the neural mechanisms that predict and reflect the development of *bona fide* anxiety and depressive disorders in highly anxious girls as they enter adolescence. Those girls identified in Aim 1 that develop stress-related psychopathology will be the focus of this aim. Based on analyses of our longitudinal WISFAM dataset, we expect that half of the ~60 girls who maintain high or worsening anxiety as they transition to adolescence will develop anxiety and/or depressive disorders during the study period (~n=30). To identify girls who develop psychopathology, anxious girls will have yearly KSAD-S interviews by [name] with diagnoses confirmed by [name]. For ethical reasons, between study visits parents will be advised to contact us if children's symptoms significantly worsen or become clinically significant. Immediate consultation with a clinician will be available, and girls who require treatment will be referred to our clinic. A final neurobiological assessment will be performed as soon as possible and will conclude their involvement in the study. Dimensional variations in sustained anxiety, worry, depression, temperament, externalizing features, and intolerance of uncertainty will be assessed with a comprehensive battery of child, parent, and clinician report measures (details in Methods).

In Aim 2, we will compare brain activation from the ~30 girls who develop anxiety and/or depressive disorders with both the other ~30 anxious girls from Aim 1 who do not have a disorder, and the ~80 girls whose anxiety-levels decreased with maturation. We are particularly interested in BST responses to uncertainty, as many patients with anxiety and depression are highly sensitive to the uncertain likelihood, quality, and/or timing of negative events. Our previous studies of > 30 un-medicated pediatric patients (age 8-12) and matched controls demonstrate that children with anxiety disorders are particularly sensitive to uncertainty. We found that during uncertain anticipation of fearful faces, anxiety disordered children show elevated activation within anxiety-related regions including the amygdala, insula, and BST (Figure 8 [90]). These compelling preliminary data, along with clinical observations of the heightened responses in young patients' while anticipating the possible occurrence of negative events, lead us to **hypothesize that with the development of psychopathology, girls with anxiety and/or depressive disorders will show greater sustained BST activation during uncertain blocks compared to girls that, even with their high levels of anxiety, have not developed psychopathology.** To isolate the neural signature of psychopathology, analysis of variance (ANOVA) models will be used to compare the final scan from girls who develop anxiety and/or depression to: 1) matched scans from the girls with stably high anxiety that do not develop disorders, 2) matched scans from the girls whose anxiety improves with maturation, and 3) their own initial scan (which is prior to the onset of their psychopathology). Analyses will be performed matching scans on the degree of sexual maturation. **Building on our data from children with anxiety disorders, we also predict that girls who develop psychopathology will have elevated acute amygdala activation during uncertainty, relative to girls who do not develop anxiety or depression.** In addition to increased uncertainty-related activation within the BST, we predict girls who develop psychopathology will also demonstrate alterations in the broader anxiety network. For example, it has been proposed that the increased incidence of stress-related psychopathology seen in adolescence is due to an imbalance between emotion processing regions of the limbic system and regulatory frontal regions [16]. Therefore, we will also explore the relations between sustained BST activation during uncertain blocks with the integrity of BST-PFC structural and functional connectivity (both at rest and during uncertainty). In addition to comparing girls with and without diagnoses, we will also explore individual differences using multimodal imaging measures, symptoms, and physiological measures. This will allow us to examine the relations between specific symptoms that are characteristic of stress-related psychopathology across diagnostic boundaries.

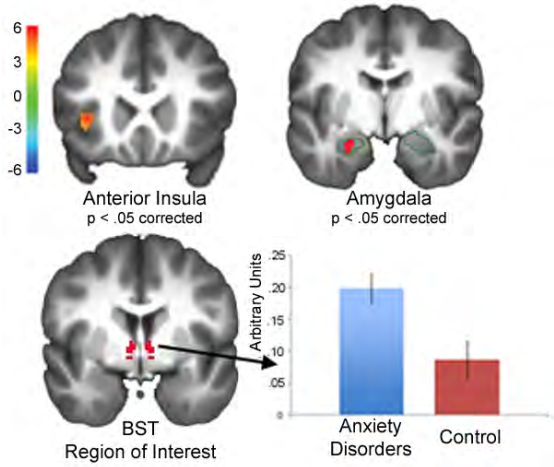

Figure 8: Regions of elevated activation during uncertain anticipation in preadolescent (8-12) children with anxiety disorders, including insula, amygdala, and BST ([name]).

Aim 3: Causal Role of the BST. Establishing the mechanistic role of the BST in highly anxious preadolescent female monkeys. Because mechanistic studies directly manipulating the function of brain circuits cannot be performed in humans, causal evidence must be derived from valid animal models. Using our well-validated nonhuman primate model of anxious temperament (AT) that is highly convergent with extreme

childhood anxiety, we will perform mechanistic studies examining the role of the BST. These experiments, complementing the longitudinal studies of anxiety trajectories in young anxious girls, will test the hypothesis that in preadolescent female monkeys, the BST plays a causal role in mediating dispositional anxiety. In addition to understanding the influences of BST lesions in primates, this study will provide a critical link between the mechanistic rodent data [45] and imaging studies of human anxiety. In preadolescent anxious female monkeys, we will use already established methods to lesion neurons within the BST [91, 92]. MRI-guided convection enhanced delivery (CED) of ibotenic acid, a fiber-sparing neurotoxin, will be used to selectively lesion BST neurons. Using ibotenic acid is critical for isolating effects to BST neurons because ibotenic acid will spare the fibers that pass through the BST, which include amygdalofugal axons *en route* to brainstem and hypothalamus. In addition to examining the effects of BST lesions on anxiety, combining the lesion strategy with multimodal imaging will allow us to understand the role the BST plays in modulating the function of other brain regions that contribute to the anxious phenotype. The acute (3 months post-lesion) and long-term effects (1 year post lesion) of BST lesions on anxiety and neural function will be studied. Understanding the long-term effects of decreased BST function in the nonhuman primate model will be important in considering the possibility of developing novel, BST-focused, anti-anxiety treatments for humans. Nonhuman primate developmental model of childhood sustained anxiety: The monkey AT phenotype models the early-life risk for psychopathology, and is assessed during the potentially threatening no eye contact (NEC) condition of the human intruder paradigm [93, 94]. As in our previously published work, freezing, reductions in coo vocalizations and increased plasma cortisol levels in response to NEC will be measured, and AT will be calculated as the mean of these 3 z-scored variables [49, 50, 94]. AT reflects a broad context-independent anxious disposition that is stable over time [47, 50, 95], predicts brain metabolism across different contexts [47], as well as other anxiety-like behaviors, including “calls for help” during separation ($r=-0.52$, $p<0.001$), aggressive barking in response to direct eye-contact ($r=-0.25$,$p<0.005$), and the latency to reach for a food reward during exposure to a novel but neutral object ($\rho=0.43$, $p=0.01$). The AT construct is therefore a context-independent, multi-dimensional phenotype similar to that observed in anxious at-risk children. As in children [46, 96-99], monkey AT is identifiable early in life, stable across development, and heritable [47, 49, 95]. Moreover, in a sample of 592 monkeys, we observed a significant relationship between early-life AT and glucose metabolism in the BST, as well as in the periaqueductal grey, a major downstream target of the extended amygdala, and in the Ce, anterior hippocampus, and caudal orbitofrontal cortex (OFC) (Figure 1), all of which are upstream modulators of the BST (Figure 9A). Collectively, these data underscore the validity of the monkey AT model, implicate the BST (and associated circuits) in early-life anxiety, and reiterate the relevance of monkey AT to the childhood risk to develop anxiety and depressive disorders examined in Aims 1 and 2.

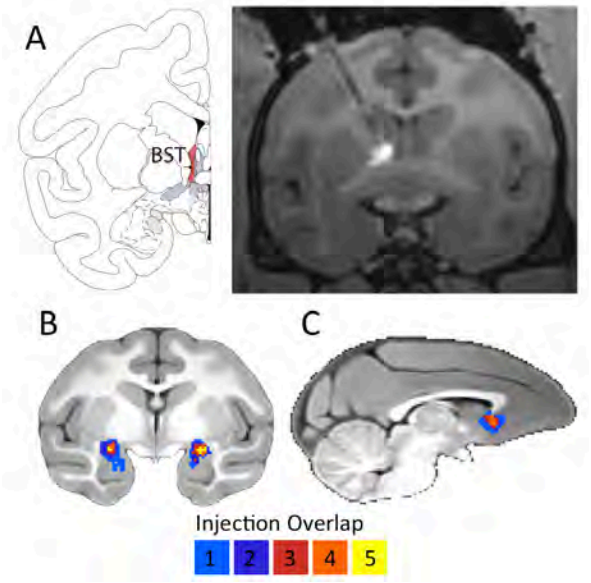

Figure 9: Data demonstrating the accuracy of the MRI-guided CED procedure. Monkey brain atlas image indicating the location of the BSTL (A, left; red). Infusion of ibotenic acid and gadolinium (Gd) can be seen within the BST (A, right). The infusion is visible on the MRI because a small amount Gd (a contrast agent) was added to the infusate (as seen in white). Below, the accuracy of our MRI guided procedure is apparent from our work infusing viral-vectors along with Gd into (B) the Ce and (C) subgenual PFC.

An important advance has been our ability to examine the neural underpinnings of AT using a combination of selective lesions and brain imaging [46, 49, 100, 101]. Our imaging data demonstrates the likelihood of multiple interacting components being involved in mediating AT (Figure 1). To date, we have mechanistically examined the role of OFC (architectonic areas 11-14) and Ce, demonstrating that lesions in these regions attenuate but do not abolish AT [91, 102]. Furthermore, evidence from our work, as well as other studies, suggests that OFC and Ce may modulate anxiety via influences on BST [102-105]. For example, using FDG-PET to study 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 BST, which remained predictive of individual differences in anxiety [103]. It is important to emphasize that no studies have been performed in primates examining the effects of BST lesions on anxiety or distributed brain function. **We hypothesize that selective lesions of the BST in highly anxious preadolescent female monkeys will diminish AT-related responding to the sustained potential threat of the human intruder during the uncertain and potentially threatening NEC context when tested 3 months post-lesion, and that these effects will be maintained as the animals develop (1-year post lesion).** Furthermore, we will examine the possibility that BST lesions will result in changes in long-range connectivity as evidenced by alterations in functional (rsfMRI) and structural (DWI) connectivity. Finally, post-lesion FDG-PET scans will allow us, for the first time in primates, to identify regions that are functionally modulated by BST input. Experimental Design**:** 100 preadolescent female rhesus

monkeys (between 1-2 years old) will be screened twice, approximately one month apart, with the NEC paradigm, and the 20 animals with the highest stable AT scores will be selected for further study. Ten of these will undergo BST lesions; the remaining 10 monkeys will serve as un-operated controls. As in our previous lesion studies, to conserve primates and cost, un-operated controls will be used instead of sham controls [91, 102]. The lesions will be made using MRI-guided CED, which includes: craniotomy and brain port placement, catheter trajectory planning, catheter insertion monitoring, real-time infusion monitoring, and post-infusion evaluation. Figure 9A demonstrates our ability to selectively target the BST with the MRI-guided technique. In conjunction with the laboratory of Dr. [redacted] we have performed over 15 CED surgeries during the past 1.5 years. We have considerable experience with selective ibotenic acid lesions, which we have performed in numerous monkeys [91, 92]. Figures 9B&C demonstrate our ability to reliably and precisely target small structures in the monkey brain using the MRI-guided surgical protocol. The primate BST is a complex structure with multiple subregions. The lateral division of the BST (BSTL) corresponds to the BST component of the central extended amygdala that in rodents is mechanistically linked to anxiety-like responding. Our lesions will target the BSTL regions that surround the anterior commissure, medial to the internal capsule and ventral pallidum, realizing that other subregions of the BST will also be damaged (see Figure 9). Initially, 2 pilot animals, that would otherwise be euthanized, will be used to optimize the injection parameters (number and volume of injections) that will produce maximal BSTL destruction while minimizing damage to nearby structures. Because the BSTL is relatively small (approx. 2 mm in the AP plane and 3 mm in the DV plane), we anticipate that 2-5 infusions (3µl per infusion) per side of ibotenic acid (1mg/100µl saline) that are placed immediately around the anterior commissure will cover the structure. Lesion animals will receive bilateral BSTL injections while undergoing real-time targeting in the MRI. To estimate infusion extent and location in real time, the ibotenic acid will be mixed with the contrast agent gadolinium (Gd, 0.66 mM). Our lab has extensive experience using Gd to ensure the accuracy of infusions as shown in Figure 9. AT, neural function (FDG-PET, rsFMRI), and neural structure (DBM, DWI) will be assessed before surgery and twice after surgery (at 3 months & 1 year) for experimental animals, and at the same intervals for the controls. A 2 by 2 ANOVA will be used to examine the effects of group (experimental vs. control) and time (post lesion vs. pre lesion) on AT and imaging measures. Other anxiety-related behaviors (the Alone and Stare conditions of human intruder paradigm, exposure to a novel conspecific, and unconditioned snake fear) will also be assessed. Blood will be collected to assess plasma stress hormone levels, as well as CSF to measure levels of corticotropin releasing hormone (CRH) and oxytocin. The use of various anxiety tests will allow us to assess the extent to which the effects of the BSTL lesions are selective to the sustained anxiety responses that are associated with AT, or more generally affect diverse fear- and anxiety-related behaviors. **We predict that BSTL lesions will have prominent effects on the sustained anxiety characteristic of AT, but will have minimal effects on the acute innate fear response elicited by snake exposure.** Because of the proximity of the BSTL to other structures, the lesions will inevitably extend beyond BSTL and may damage other BST regions and cell groups of the basal forebrain. Lesioned animals will be sacrificed at the end of the experiment for analysis of lesion effects in relation to the extent of BSTL tissue destruction and collateral damage.

Aim 4: Molecular alterations in BST neurons. Examining gene expression in BST neurons from preadolescent female monkeys in relation early-life anxiety and BST metabolism. Understanding the molecular processes within the BST that lead to dysregulated BST function in highly anxious young females will begin to define molecular mechanisms underlying anxiety. This has the potential to guide the development of novel treatments aimed at preventing further suffering during the transition to adolescence. To this end, we will identify alterations in gene expression by performing RNA sequencing on BST tissue from the brains of 24 young female monkeys that have already been collected and are part of our non-human primate brain bank (Age: mean=2.2 years, sd=0.51). Each animal was fully phenotyped for AT and with our multimodal imaging and behavioral measures described in Aim 3. In addition to understanding alterations in gene expression that are relevant to variation in anxiety, these studies will provide an important first look into the molecular substrates of primate BST function. To ensure that our RNAseq data reflects neuronal function, we will use LCM to specifically collect neurons from BSTL (described below). Individual differences in gene expression will be analyzed in relation to both BST metabolism and AT. Although our imaging methods cannot distinguish between medial and lateral BST, our focus will be on the BSTL, because: i) the lateral BST is implicated in rodent anxiety, ii) compared to the medial BST the BSTL is relatively enlarged in primates, and iii) the BSTL contains similar neuronal subtypes as those seen in the central nucleus of the amygdala (Ce) [106].Our recent work has examined gene expression and epigenetic modification within the Ce in young non-human primates, and the data from these studies will guide our hypotheses for the proposed study. ***We predict reduced expression of specific neuroplasticity genes in young female monkeys with high levels of BST metabolism and increased AT. Additionally, we will use a discovery-based approach to identify predicted and unpredicted transcripts that relate to BST-metabolism and AT.*** Our Ce microarray expression studies in 24 preadolescent monkeys implicate predicted genes (e.g. NPY1R, 5-HT2c) as well as neuroplasticity-related molecular alterations associated with AT and increased Ce metabolism (Figure 10).

Within the Ce the NTF3 (neurotrophin-3)-NTRK3 pathway was most robustly involved. NTRK3 (neurotrophic tyrosine kinase receptor-3, also termed TrkC) is of particular interest because its activation can initiate synaptogenesis and neurogenesis [107].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 [108, 109]. Importantly, our more recent studies have examined gene expression and DNA methylation in an independent set of 24 young rhesus monkeys, and have similarly implicated decreased expression and increased methylation of neuroplasticity genes in highly anxious young monkeys (Alisch et al., under review). Because of the similarities in neuronal composition between BSTL and Ce, we hypothesize that we will identify similar alterations in BSTL expression that are relevant to AT. Like the Ce, the BSTL is comprised of predominantly GABAergic neurons [106]. We will use LCM techniques that are currently being used in our lab to dissect individual neurons from the Ce (Figure 11). Already collected fresh frozen brains will be sliced throughout the entire extent of the BSTL (14 µM sections). For LCM, we will rapidly stain BSTL neurons with a NeuN antibody to guide selective neuronal dissection with our Leica LMD6500 scope (Figure 11). In our collaboration with the [name] laboratory, we have determined that 750 cells are optimal for our analyses. RNA and DNA will be extracted from harvested neurons. Gene expression will be quantified using RNAseq methods already established in our collaboration with the [name] laboratory. BSTL DNA will be stored for future epigenetic studies. Alternate sections through the BST will be used to subtype the BSTL GABA neurons using immunohistochemical techniques established in our laboratory in conjunction with the UW Cellular Neurobiology Core (e.g. somatostatin, CRH, etc.). This information will guide subsequent experiments aimed at examining altered gene regulation in specific BSTL neural subtypes. RNAseq will be performed using Illumina HiSeq DNA sequencers and cutting-edge mRNA sequencing methods (see Detailed Methods). Analyses will use our own RANseq workflow, which is based on RseqFlow [110] and supported as part of the iSeqTools Network (NHGRI 1U01HG006531, [name] Co-PI), to characterize the transcriptional structure of genes, including their splicing patterns and other post-transcriptional modifications, as well as quantify gene expression and splice-variants. Initial RNAseq analyses of Ce data from 47 monkeys demonstrate the feasibility of our collaboration with the [name] group and the value of using RNAseq. Consistent with human work, our RNAseq and microarray measures from the Ce tissue were highly correlated (*r*s>.72). Importantly, qRT-PCR on divergently expressed transcripts found higher convergence between PCR (the "gold standard") and RNAseq (R^2=.93) than between PCR and microarray (R^2=.71). The Kalin-[name] collaboration has already produced over a billion RNAseq reads from the Ce of 47 animals, putting us at the forefront of non-human primate transcriptome analyses. Using currently available and developmental builds of the rhesus monkey genome, we are currently mapping ~70% of reads, and will continue to improve our techniques as new strategies and reference data become available. Quantification of gene expression patterns in the Ce reveal robust correlations across individuals (r's >.9), demonstrating technical stability. Moreover, an initial comparison of Ce gene expression from these 47 animals in relation to AT monkey revealed numerous differentially expressed genes, suggesting this technique is sensitive to individual differences. Excitingly, the differentially expressed genes include genes that predicted AT in our earlier microarray work (e.g., RPS6KA3, also known as RSK2, which has been implicated in controlling cell growth and differentiation). An additional opportunity of this proposal will be the ability to compare RNA expression profiles between BSTL and Ce. Though the Ce-BSTL similarities motivate our gene expression hypotheses, there are important functional differences between these regions. Thus,

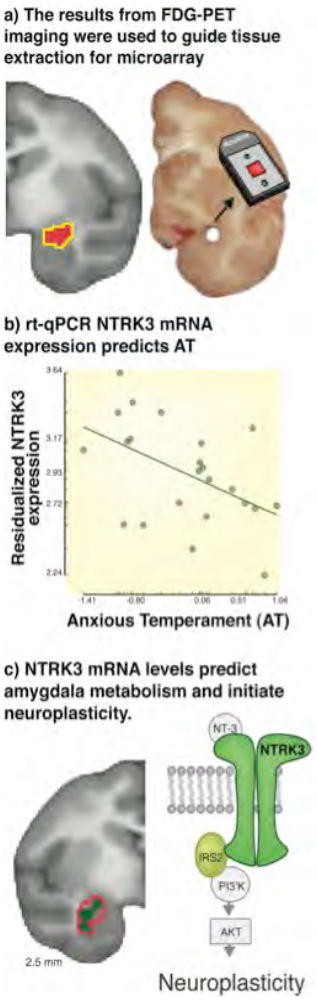

Figure 10: ***Ce expression of the neurotrophic receptor NTRK3 predicts AT*** **(A)** Statistical parametric map depicting correlations between brain metabolism and AT (left) was used to guide biopsies of Ce tissue, which was used to identify AT-related transcripts in our microarray study (right). (**B**) rt-qPCR confirmation of the NTRK3-AT relationship observed using microarray ($p<0.05$). **C)** Statistical parametric map showing the significant correlation between NTRK3 mRNA expression and Ce metabolism *in vivo* (green; $q<0.05$, corrected) (**left**). The NTRK3 pathway associated with increasing neuroplasticity (**right**).

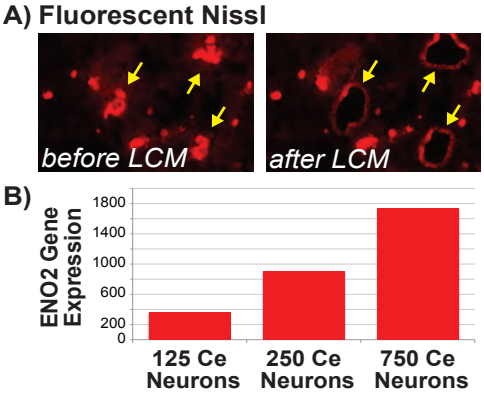

Figure 11: A) Ce Neurons before (left) and after LCM (right). B) Neuron specific enolase (ENO2) expression from various numbers of LCM dissected neurons from Ce.

combination of the resulting BST-RNAseq dataset with our existing Ce-RNAseq dataset will allow us to understand anxiety-related patterns of BST gene expression that are common and distinct from those observed in the Ce.

Methods: We have published extensively on many of the methods discussed and cited 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 and Affiliate Scientist at the Wisconsin National Primate 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 adults and children with anxiety and depressive disorders. To support the integrated goals of this translational study, Dr. Kalin has assembled a team of expert collaborators. The team has expertise in longitudinal studies of health, emotion and behavior ([name redacted] & Kalin), and the clinical characterization of children with anxiety disorders [name redacted] &Kalin); as well as experience with neuroimaging of children and adolescent's that is crucial for Aims 1 and 2 ([name redacted] & Kalin). In addition, several members of the study team have worked together for many years on studies of non-human primate anxiety ([name redacted] & Kalin) and non-human primate brain imaging ([name redacted] & Kalin). More recently we have developed sophisticated protocols for MRI-guided targets of small brain structures that will be utilized in the lesion studies for Aim 3 ([name redacted] & Kalin). Finally, our own expertise in primate anatomy and LCM ([name redacted] & Kalin) will allow for dissection of BST neurons for RNA sequencing in collaboration with experts in the field ([name redacted] & Kalin). **Longitudinal study of anxious girls as they transition to adolescence.** Recruitment will be informed by Dr. [name redacted] extensive experience with longitudinal studies [77]. As is standard in the Kalin and [name redacted] labs, newspaper ads, mass emails, and letters distributed through elementary schools in the Madison metropolitan area will be used to inform girls and their families about the study. Children and their parents will be asked to complete the SCARED at two time-points ~1 month apart, as well as an eligibility questionnaire, via an online interface to determine initial eligibility. Highly-anxious girls meeting inclusion/exclusion criteria will be paid $200 for participation in each set of study visits (K-SADS, MRI, EMG) plus $50 for TSST study visit at initial assessment. An additional incentive ($100) will be offered to girls who complete all study visits they qualify for; girls who end their participation due to need for treatment will be paid this incentive. The *entry and inclusion criterion* are 1) female, 2) age 10-11, and 3) scores of >10 on two assessments (~1 month apart) with the Parent-SCARED scale. The *exclusion criteria* are: 1) diagnosis or history of any DSM-V disorder at study entry; 2) history of brain damage or significant developmental delay (IQ<80); 3) acute and/or unstable medical illnesses; 4) chronic medical illness requiring medication treatment other than allergies such as hay fever; 5) psychotropic drug use within 2 weeks or if previously on fluoxetine within 4 weeks; 6) taking medications known to affect autonomic and/or CNS function; 7) use of oral steroids; 8) metallic implants; and 9) claustrophobic or unable to stay still in the MRI scanner. *Study visits at each phase of testing: initial assessment, 1 year later, 2 years later, and 3 years later.* All methods described above have been performed in our laboratory with 8-12 year old children with and without anxiety disorders. Each phase of testing will involve three study visits (~2 hours each); the initial study assessment will include a 4th visit for the TSST. To control for time of day, especially in relation to cortisol sampling, all study visits will be between 2 and 5 PM. At **Visit 1**, Dr. [name redacted] will perform a K-SADS, which will be followed by training in a mock MRI scanner and IQ assessment using the WASI. K-SADS interviews will be audiotaped and sent to [name redacted] for diagnosis confirmation as we have done in our current pediatric anxiety study. At the beginning of **Visit 2** diagnoses will be confirmed by Dr. Kalin and girls will complete a refresher mock MRI scan. Two 1.5 ml saliva samples will be collected at 3 times (after study arrival ~2:30 PM, break during MRI scan ~3:30 PM, end of visit ~4:30 PM) for assessment of change in cortisol across the session and for measurement of estradiol and testosterone. Per our lab protocol salivary samples will be stored at -80°C and assayed with our routine and well-established methods [85]. Buccal swabs will also be collected and stored for future genetic analyses to begin building a DNA database from preadolescent girls with high levels of anxiety. Between Visits 1 & 2 parents and children will complete clinical rating scales listed below. The MRI scan will be broken into two 25 min parts separated by a 10 min break. MRI scans will collect structural MRI (T1), resting functional connectivity (fMRI), countdown task (fMRI), and DWI data. At **Visit 3**, girls will complete the startle potentiation to acute and sustained threat. Parameters will be identical to the protocol published by Grillon [89], with the exception of using a mildly aversive, child-appropriate [111, 112] threat, a 100 ms blast of air to the neck at 60 PSI, rather than a shock. At the initial assessment during **Visit 4** girls will complete the *Trier Social Stressor Test (TSST)*. We will administer and score the TSST as in our previously published work [88], with the exception that cortisol will be sampled after a 60 minute acclimation period (baseline), immediately after, 30 minutes, and 60 minutes after the TSST. After the TSST girls will complete a writing exercise previously shown to boost self-esteem and will be thoroughly debriefed. *Clinical Measures*: At each phase of testing multiple measures will be collected to quantify participant's symptoms from several viewpoints, including: 1) anxious girls (SCARED-Child, CDI, Positive and Negative Affective Scale-Child (PANAS-C), IUSC, PSWQ-C, TMCQ), 2) parents of anxious girls (SCARED-Parent, Connors' Parent Rating Scale-Revised: Short Form (CPRS-R:S), and 3) assessment by Dr.

name (PARS, CGI, Global Assessment Scale for Children (CGAS)). *Developmental Measures*: Individual differences in sexual maturation will be assessed annually at each K-SADS visit using Tanner ratings [113], which will be included with pubertal hormones in growth curve analyses as indicators of maturation. We collected these measures in our studies of childhood anxiety and found, as expected, that hormone levels correlated with both age and Tanner ratings. *Countdown task*: This task is currently in use in the name laboratory, and is a modification of her published sustained BST activation paradigm in adults [55]. For details see Approach section Aim 1 and Figure 5. At the beginning of each block participants will be informed of the block type (e.g. Neutral pictures with Certain Clock). Girls will complete two blocks of each condition (negative picture certain timing, negative picture uncertain timing, neutral picture certain timing, neutral picture uncertain timing) across 4 functional runs. Fixations will be presented for 30-seconds at the beginning, middle, and end of each block to provide a baseline for analysis. **MRI Parameters and Analysis for girls and monkeys**: Anatomical scans and resting state data for both species will be collected and analyzed as described in our monkey-child anxiety translational MRI study [84] using an anatomical BST seed. Countdown task data will be collected as in [84], preprocessed as in [114] and analyzed as in [55]. To account possible differences in brain volume (e.g. [115]), functional analyses will use individual differences in DBM measures (log jacobian determinant) as voxelwise covariates. Psychophysiological interaction analyses will be performed on task data for each condition using a BST seed. *DWI Parameters and Analysis*: The integrity of white matter pathways passing through the BST (e.g. the stria terminalis) will be evaluated with DWI data collected using a two-dimensional EPI diffusion-weighted spin-echo sequence (TR/TE/Flip/Matrix: 6500ms/62.2ms /90/256x256; 2.9 mm contiguous slices; echo-planar echo spacing = 568us; b=1000 s/mm^2; 48 non-collinear directions; 8 non-diffusion weighted images). Brains will be normalized using DTI-TK, which iteratively constructs a template from the tensor files as in [116]. FA and other scalar maps will be computed and tractography will be performed using Camino. **Selected methods for establishing the causal role of the BST in extreme early life anxiety via BST lesions in young anxious female monkeys**. The MRI-guided CED surgery methods that are featured in Aim 3 were developed based on the work of our collaborator at the Wisconsin National Primate Center, Dr. name [117]. Methods have been published for our behavioral and hormonal assessments of AT, FDG-PET imaging methods, lesion methods, post-mortem histology, euthanasia methods, and other anxiety-related measures. These procedures will be performed in accordance with our standard lab protocols [46, 48-50, 66, 91, 94, 95, 102, 103]. Design of the lesion experiment is described in Aim 3; assessment of AT and multimodal neuroimaging will be conducted at 3 time points: prior to surgery, 3 months after BST lesion, and 1 year after BST lesion. **Detailed methods for Aim 4:** LCM: 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 BSTL neurons from cryostat-cut tissue sections stained with neuronal markers and mounted on slides that are viewed under a microscope. Landmarks for BSTL will be determined from alternate 14 µM sections (every 6th section) stained for AChE, which allows for the identification of BSTL. BSTL neurons will be identified using the NeuN antibody, which allows for rapid staining with preservation of RNA. Dr. name who is an expert in the anatomy of primate BST and has extensive experience with the Leica LMD6500 scope, 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 BSTL 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. Pools of approximately 750 neurons collected from the BSTL will be studied using RNA-Seq in the Knowles laboratory. We propose to use a modification of the SPIA reaction of the NuGEN Ovation RNAseq V2 kit for cDNA Synthesis, followed by library construction using the NuGEN Rapid no-PCR protocol in a Mondrian microfluidics instrument. Unlike the Clontech SMARTer Ultra Low Input RNA kit we used previously (Qiu et al., 2012), 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. Our protocol uses linear amplification and amplifies the nucleic acid ~1,000X by the end of library construction, as compared to ~2.6 million-fold in the SMARTer-TruSeq protocol. 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 r2 of the 28 pairwise comparisons was 0.59, and ~15,000 transcripts were detected in each library (UHR comes from 10 cell lines and is a more stringent test than single cell RNA which only has only 5-7,000 transcripts). When the same experiment is performed using 100pg (~10 cells) or 1ng (~100 cells), the average r2 of the pairwise comparisons was 0.84 and 0.92, respectively. Read Mapping And Determination Of Expression Levels. The above data are analyzed with our own RNA-Seq analysis pipeline, GT-FAR (Genome- and Transcriptome-Free Analysis of RNA-Seq). GT-FAR is the next iteration of RseqFlow (Wang et al., 2011) and is supported by NHGRI as part of the iSeqTools Network (1U01HG006531; Knowles Co-PI). 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 or 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 human transcriptome (presently GenCODE v 19) 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 genome (hg19), without, and with, gaps, often finding novel exons or transcription units. From some human samples from cell lines we have mapping rates as high as 99%, leaving only 1% of reads unaccounted for. For RNA-Seq data from Rhesus, we are currently mapping ~70% of reads to the Rhesus transcriptome and genome. **Analysis, power and sample size. *Power calculations for all studies*:** 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) [124], we calculated power analyses using α=0.005, the 2-tailed, single-comparison alpha needed to achieve FDR q=0.05 across comparisons. **Growth curve analyses for Aims 1 and 2:** To compare developmental trajectories between girls who maintain stable or have increasing anxiety over the study period and those girls who naturally improve with maturation, in collaboration with [name redacted] we will use a latent growth curve analysis within a hierarchical linear modeling framework. This approach is ideal as it allows for the simultaneous estimation of group level and individual subject patterns, the inclusion of unequally spaced time points and partially missing data, (i.e. girls who do not complete all study visits because they develop psychopathology and require treatment). Subjects who complete at least 1 assessment will be included. Change over time for primary neuroimaging outcome measures, e.g. sustained BST activation to negative pictures, will be compared between groups. Age, hormone levels, and Tanner stage will be included as covariates. Slope estimates will be allowed to vary freely across participants. If variability in slopes is significant, the relationship between slopes and other predictor variables (slopes as outcomes model) will be examined. While we predict relatively linear changes in sustained BST activation over development, polynomial functions will also be considered. Model fits will be evaluated using maximum likelihood estimation. ***Sample size Aims 1 and 2: Anxious girls.*** Estimation of effect sizes for differences in brain activation between groups are based on our previous studies of preadolescent children with anxiety disorders, where we have observed large effect sizes during uncertainty (d=0.87) [90] and in at rest (PFC-amygdala connectivity d=.78) [84]. Sample sizes were selected to provide at least 70% power to detect similar large effect sizes. 200 girls will be enrolled to account for exclusions (current psychopathology) and attrition in longitudinal follow-up (Figure 4). **Aim 1:** We will test the hypothesis that girls who remain highly anxious as they transition to adolescence will show stable or increasing levels of BST activation, whereas girls who improve will show decreasing sustained BST responses. We will have >80% power to detect large effects (d=0.8) for a linear change model (Calculated using Optimal Design Software [125], n=140, frequency=4, duration=3 years). **Aim 2:** ANOVA models will be used to compare the final scan from girls who develop psychopathology to 1) girls who maintain high anxiety but do not develop psychopathology, 2) girls who recover with maturation and 3) to their own baseline scan. The proposed sample sizes will yield > 75% power to detect large (d=0.8) effects (n=30/group, df=55, calculated in GPower V 3.1.9.2). Correlational analyses will examine the relationship between specific clinical and physiological variables (e.g. intolerance of uncertainty and cortisol) with specific neuroimaging measures (e.g. sustained BST activation during uncertainty). ***Sample size Aim 3: BST lesions***: Based on our previous lesion studies the effects of BST lesions on AT are expected to be substantial (d=1.8). The proposed 10 subjects/ group will yield > 85% power to detect similar effects (n=10/group, df=17, calculated in GPower V 3.1.9.2). A 2 by 2 ANOVA will be used to examine the effects of group (experimental vs. control) and time (post lesion vs. pre lesion) on AT and imaging measures. ***Sample size Aim 4: RNAseq from BSTL neurons***: Based on our previous microarray [95, 126] and RNAseq data we expect a strong relationship between AT, BST metabolism, and variations in neuroplasticity genes (f^2=.35), which will give us > 75% power to detect effects of this size with 24 subjects (n=24, df=21, calculated in GPower V 3.1.9.2). Differences in transcript expression levels from BST neurons will be analyzed in relation to AT and BST metabolism during the NEC condition. Primary analyses will use a massively univariate approach and standard multivariate regression techniques that will control for potential confounds (e.g. age and gray-matter probability [127]) and use FDR-techniques (q<.05) to correct for multiple comparisons [128]. Because we expect a large number of null results in transcripts and voxels unrelated to AT, we will incorporate the empirical distribution of these null findings into our probability estimation using empirical Bayes techniques [129]. 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 RNAseq findings using GO enrichment, weighted co-expression gene network, Ingenuity Pathway analyses. **All Aims: Multiple comparison corrections for neuroimaging data:** Within each Aim we have primary hypotheses that focus on the role of the BST in sustained anxiety. In these initial planned comparisons we will correct for multiple comparisons using Šidák techniques, and will consistently achieve > 95% power to detect moderate effects (d>.6). 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, corrected for multiple comparisons. This approach allows us to correct for multiple voxelwise analyses with moderate power as detailed above.

Projection of Human Subjects

Risks to the Subjects:
Human Subjects Involvement, Characteristics, and Design: This structural and functional MRI and DWI protocol will require MRI scanning of roughly 170 girls between the ages of 10-14 years over the five years of the project. Girls will be recruited from the Madison metro area via advertisements and will have high levels of anxiety but will not be diagnosed with any DSM-V diagnoses at study entry. Sources of Materials: The sources of the research materials will include image data collected from the MRI scans from this study. We will also use endocrine, physiological, behavioral and clinical measures. Saliva samples will be used solely for analysis of hormone levels. All subject information will be kept confidential and names or other identifying codes will not be used in reporting the data. Potential Risks: Subjects will be screened for contraindications for MRI (e.g., metal or electronic implants, severe claustrophobia, etc.). If any of these conditions are found, the subject will be excluded from the study. The risk associated with the MRI procedure is minimal. We screen each subject three times before every MRI visit - over the phone, when they arrive at the lab for the scan on Visit 2 and a third time before they enter the scanner room (the last screening a standard procedure of the scan technicians, and is primarily for metal objects on their person). If any contraindications are found, the subject will not be scanned and they will be excluded. Hearing protection (either headphones or earplugs) will be used to reduce the apparent scanner noise level and to increase subject comfort. Individuals with claustrophobia may experience some anxiety in the confined bore of the magnet.

Adequacy of Protection Against Risk:
Recruitment and Informed Consent:
Girls will enter the study on a voluntary basis. The details of the MRI study will be discussed with each subject and they will be required to read and sign an informed consent form for the MRI procedure with the help of their parents. Girls who require treatment for DSM-V diagnoses will not be enrolled in the study.

Protections Against Risk:
Every effort will be made to provide information to the subjects to minimize anxiety about the imaging procedures. All procedures will be performed in accordance with HIPAA regulations. Subject confidentiality will be maintained throughout the project period. Names will not be kept on data records and a standardized code will be used for subject identifiers. Subjects will only be identified by a code number (including the MRI image studies). Any records with subject specific information will be kept in a locked file. Parents will be informed of adventitious behavioral/psychiatric findings determined by the trained clinician to be of significant safety concern. A Certificate of Confidentiality will be applied for through the NIH in order to further protect the confidentiality of the subjects. To ensure participant safety during the study period, they will have regular assessments, including self-report measures of anxiety and depression and clinical interviews with study psychologist [redacted] If girls develop psychopathology that requires treatment, they will be prematurely terminated from the study and referred to treatment.

Potential Benefit of Proposed Research to the Subject and Others:
The risks to the subjects in this study are minimal. The results from this study will benefit the clinical, imaging and neuroscience research communities through publications regarding the results of our comparisons, and through the new techniques that are developed in the project. The anonymous image data sets will be made available to the research community at the conclusion of the study. The potential benefits of this study far outweigh the minimal risks involved.

Importance of Knowledge to Be Gained:
In this study, we will utilize a set of advanced imaging techniques to study brain response to sustained threat, as well as resting functional and structural connectivity in a clinically characterized sample of highly anxious girls as they transition to adolescence. Trajectory analyses will allow for a comparison between girls whose anxiety naturally improves with maturation, those who remain anxious, and those who develop psychopathology. These data have the potential to identify novel markers of risk and resilience in this vulnerable population who are at an increased risk to develop anxiety and depressive disorders.

Inclusion of Women and Minorities

Persistent and high levels of sustained anxiety during childhood are a strong predictor of the development of anxiety and depressive disorders during adolescence. This is particularly relevant to females because after puberty girls are twice as likely to develop these disorders. To understand this increased risk of stress-related psychopathology for girls as they enter adolescence, these studies will focus exclusively on females during this vulnerable transition. Additionally, the bed nucleus of the stria terminalis (BST), which is our primary brain structure of interest, begins to exhibit sexually dimorphic features during adolescence in parallel with increased rates of anxiety and depression in females. A sufficiently large sample of anxious girls and anxious female monkeys, as we propose to collect in this study, is necessary to 1) adequately characterize BST development as anxious females transition from late childhood to adolescence, 2) understand the relation of BST responses during sustained anxiety to the development of psychopathology, 3) test the causal role of the BST in anxious behavior and 4) examine the molecular underpinnings of BST activation and anxiety.

Minority group members will be recruited in approximately proportion to their representation in the state/local population. Approximately, 10% of the Wisconsin state and Dane county populations are in a racial minority. Accordingly, we will recruit a minimum of 10% minorities into the study. The minority breakdown of Dane County is roughly 90% Caucasian; 4% African American; 3% Asian American; and 3% other.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Extreme anxiety in females: the role of the bed nucleus of the stria terminalis (BST)

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	2	0	0	0	**2**
Asian	4	0	0	0	**4**
Native Hawaiian or Other Pacific Islander	2	0	0	0	**2**
Black or African American	4	0	2	0	**6**
White	176	0	4	0	**180**
More than One Race	4	0	2	0	**6**
Total	**192**	**0**	**8**	**0**	**200**

Study 1 of 1

Funding Opportunity Number: PA-13-302. Received Date: 2014-10-03T16:56:13.000-04:00

Inclusion of Children

Females age 10-11 will be eligible for study enrollment, and will be followed up to age 14. The goal of the proposed research is to use multimodal brain imaging to study girls with high levels of anxiety as they develop from late childhood to adolescence. This transition, particularly in females, is a key time for the emergence of anxiety and/or depressive disorders. Studying the neural correlates of anxiety symptoms in young females will provide information less complicated by chronicity and possible treatment effects than similar studies of adults or adolescents. To accomplish this, children must be enrolled. The selected age range captures the end of childhood (age 10-11) and follows girls into the beginning of adolescence (age 13-14).

Our research team has extensive clinical experience working with children in this age range including name [redacted] and Dr. name, an expert in neuroimaging studies of child and adolescent mood disorders. Dr. name has conducted K-SADS interviews with anxious children for more than 10 years, worked to do this with Dr. Kalin for the last 4 years. Dr. Kalin, Dr. Oler, and Dr. name have been successfully collecting neuroimaging data from children (age 8 – 12) with and without anxiety disorders for the past 4 years, demonstrating our ability to collect high-quality data from participants in the proposed age range.

The magnetic resonance imaging (MRI) facilities at the location [redacted] have several unique features to accommodate child participants. Chief among them is an MRI simulator set up, a decommissioned MRI scanner without a magnet, which is used to allow all possible child participants exposure to the MRI environment in a gradual and low-pressure manner. During the simulator session children will be placed inside the scanner tube, played a recording of the sounds of an MRI scan, and be exposed visual and auditory stimuli. Children can decide whether they want to proceed to the actual scan session before scheduling the next study visit. These MRI simulator sessions also improve data quality because children can be given feedback on motion during the practice scan, and only children with the ability to stay still will advance to the MRI portion of the study. HERI MRI staff with extensive experience in child neuroimaging from this and other active protocol. As the entire study sample is composed of child participants, the projected enrollment table shows that we will include a sufficient number of children, enrolling up to 200 young females into the study over the grant period, to perform a meaningful analysis of neural circuits associated with sustained elevated anxiety across development in females.

Vertebrate Animals

1. PROPOSED USE OF ANIMALS

To perform the proposed work aimed at elucidating the causal role and molecular mechanisms of the BST that underlie anxious temperament (AT) we will repeatedly study 20 female rhesus monkeys (*Macaca mulatta*). Ten of these animals will undergo BST lesions. To conserve animals and cost, the remaining 10 monkeys will serve as un-operated controls. We will test the hypothesis that selective lesions of the BST in highly anxious preadolescent female monkeys will diminish AT-related responding to the sustained potential threat of the human intruder during the NEC context (see below) when tested 3 months post-lesion, and that these effects will be maintained as the animals develop (1-year post lesion). Mechanistically implicating the BST in extreme dispositional primate anxiety is crucial for understanding the extent to which novel therapies to treat and prevent anxiety disorders should be aimed at altering BST function.

In Specific Aim 3, we will screen 100 preadolescent female rhesus monkeys (between 1-2 years old), and the 20 animals with the highest stable AT scores will be selected for the lesion study. Ten of these animals will undergo BST lesions using real-time MRI guided delivery of ibotenic acid (see surgery section below), while the remaining 10 monkeys will serve as matched controls. Following recovery from surgery the BST-lesioned animals (and their matched controls) will be tested using a variety of standard behavioral, brain imaging and physiological tests. Animals will be phenotyped with these measures and blood for DNA and RNA will be collected and stored for future analysis. We will also measure plasma stress hormone levels, as well as collect CSF to measure levels of corticotropin releasing hormone (CRH) and oxytocin.

In Specific Aim 4, we will use laser-capture microdissection to dissect individual neurons from the BST for use in RNA sequencing. We aim to identify alterations in gene expression by performing RNA sequencing on BST tissue from the brains of 24 young female monkeys that have already been collected and are part of our non-human primate brain bank (Age: mean=2.2 years, sd=0.51). These 24 monkeys were sacrificed as part of a different grant focused on AT-related molecular mechanisms within the central nucleus of the amygdala (Ce). Importantly, each of these 24 animals was fully phenotyped for AT and with our multimodal imaging and behavioral measures described below. This phenotyping will be used in conjunction with deep RNA sequencing to investigate the molecular mechanisms in the BST underlying the risk to develop anxiety and depression.

To assess anxiety-related behavior, the following tests will be performed: 1) 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 or conspecific. Behavior will also be assessed during the NEC and ST conditions. During the NEC condition a human intruder enters the test room, never making eye contact with the animal and stands 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. 2) Stranger Conspecific Paradigm - Animals will be re-housed with a novel animal to evaluate anxiety and arousal during social interaction. During re-housing, behavioral data will be obtained. Hormonal status will also be evaluated by measuring plasma cortisol levels. To accomplish this, blood will be sampled immediately following the novel conspecific test. 3) Snake Exposure Test - During the snake exposure test, animals will be placed in a test 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.

All imaging modalities proposed in this study are safe and routinely used in human research. To assess brain metabolism with positron emission tomography (PET), subjects will be injected intravenously with ^{18}F radioactively tagged fluorodeoxyglucose (FDG, up to 10 mCi). During the uptake of FDG, the subjects will be exposed to the NEC condition for 30 minutes. At the end of this 30-minute uptake period, subjects will be anesthetized with 15 mg/kg ketamine (IM) and blood will be sampled by venipuncture for plasma cortisol levels. Animals will then be fitted with an endotracheal tube to deliver 1-5% isoflurane gas anesthesia during the PET scan while brain metabolic activity will be imaged. At least 5 days after PET, high-resolution anatomical and functional MRIs will be acquired. These images will be used to assess variation in structural morphometry, structural connectivity and functional connectivity, and to co-register with PET data.

MRI scans are commonly used in humans and give detailed images of the anatomy of the key brain regions underlying anxiety as well as the structures connecting these key brain regions. The animals will be transported to the MRI suite in a primary transport cage within a rigid, opaque secondary leak proof container

with or without a small window for animal observation. In order to accomplish MR imaging, animals will be chemically restrained with Ketamine HCL (up to 15 milligrams/kilogram given intramuscularly), which will be repeated as needed approximately every 20-40 minutes. The initial dose will be given after the subject is positioned on a restraint table. After the initial dose of anesthesia takes effect, the monkey will be transported to the MRI suite and scanned for approximately two hours. No animal will receive more than 40 milligrams/kilogram Ketamine HCL in a single day. Up to 0.025 milligrams/kilogram dexmedetomidine will be given intramuscularly. The dexmedetomidine will be reversed with up to 0.25 milligrams/kilogram dose of atipamezole given intramuscularly. Atropine sulfate (0.01 – 0.3 milligrams/kilograms intramuscularly or subcutaneously) may be given to depress salivary secretion. Heart rate and oxygen saturation will be monitored continuously and recorded every 15 minutes throughout the scan to evaluate the depth of anesthesia, and changes in these vital signs during the scan will be used to determine if additional doses of Ketamine HCL are needed. If heart rate decreases significantly, relative to baseline, we will reverse the dexmedetomidine using atipamezole (up to 0.25 milligrams/kilogram intramuscularly). Body temperature will be measured and recorded before and after imaging and maintained by wrapping animals in bubble wrap and/or blankets. Animals will be removed from the scanner and monitored until they can be safely transported.

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

We will perform ibotenate lesions with intraoperative MRI guidance. Ibotenate injection surgeries will ablate neurons within the BST region while sparing axonal fibers passing through the BST region. This will enable us to implicate BST neurons as causally related to behaviors that increase the risk to develop psychiatric illness. 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 intercranial 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 maybe 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 BST 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 [redacted] 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 [redacted] 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 1 week before post-surgical testing will begin.

2. JUSTIFICATION OF ANIMAL USE

Rhesus monkeys serve as an excellent model of fear and anxiety because like humans they have a well-developed prefrontal cortex with extensive neural linkages between the frontal/temporal cortices and the amygdala and hippocampus. These are the same neural circuits that subserve the expression and regulation of fear and anxiety in humans. Studies of this nature are critical for 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 this type of study. The social 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 AT, including hormonal and neural measures. The data to be collected will allow us to determine the causal role of the primate BST in anxiety, and identify altered BST gene expression that may be critically involved in the pathophysiology of anxiety disorders. This is essential because: 1) it will provide insights into the different mechanisms underlying the heterogeneous expression of anxiety, and 2) it will allow for understanding the mechanisms underlying the sustained anxiety responses that are a hallmark of the risk for anxiety and affective disorders and 3) has the opportunity to provide a rationale to develop interventions targeted at specific molecular components of the BST that may be critical to helping at risk anxious children.

3. VETERINARY CARE

Veterinary care, supported by clinical laboratories, is available at the Primate Center and the Harlow Center for Biological Psychology 24 hours a day, seven days a week, by 11 veterinarians as well as 17 back-up veterinarians campus wide.

4. PROCEDURES TO MINIMIZE DISCOMFORT 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. Heart rate, respiration and oxygen saturation will be monitored throughout the PET procedure, and body temperature will be maintained using a warm air blanket. 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.

5. EUTHANASIA

To evaluate lesion extent animals will be euthanized under the guidance of veterinary staff using pentobarbital overdose, which is the standard method of humane euthanasia at these facilities. This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

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Form Approved Through 6/30/2012 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Extreme anxiety in females: the role of the bed nucleus of the stria terminalis..

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PA-13-302 Title: Research Project Grant (Parent R01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)
[redacted]

3b. DEGREE(S)
MD PhD

3h. eRA Commons User Name
[redacted]

3c. POSITION TITLE
[redacted]

3d. MAILING ADDRESS *(Street, city, state, zip code)*
[redacted]

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Psychiatry & the Behavioral Sciences

3f. MAJOR SUBDIVISION
Keck School of Medicine of USC

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: [redacted] FAX: [redacted]

E-MAIL ADDRESS:
[redacted]

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☒ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
	☒ No ☐ Yes	☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☒ No ☐ Yes — 5a. Animal Welfare Assurance No.

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
7/1/2019	6/30/2020	$47,165	$76,847	$47,165	$76,847

9. APPLICANT ORGANIZATION
Name: University of Southern California
Address: Department of Contracts & Grants
2001 N Soto Street, SSB 205
Los Angeles, CA 90089-9235

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
195-1642394A1
DUNS NO. 0729333930000 Cong. District CA-034

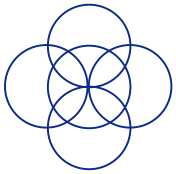
12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Linda Bakabak
Title: Senior Contract and Grant Officer
Address: Department of Contracts & Grants
2001 N Soto Street, SST 205
Los Angeles, CA 90089-9235
Tel: 323-442-3982 FAX: 323-442-2835
E-Mail: lbakabak@research.usc.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Allison Ramos
Title: Principal Contract and Grant Officer
Address: Department of Contracts & Grants
2001 N Soto Street, SST 205J
Los Angeles, CA 90089-9235
Tel: 323-442-0154 FAX: 323-442-2835
E-Mail: ramosa@research.usc.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13.
(In ink. "Per" signature not acceptable.)
[redacted]

DATE
9/30/2014



WAISMAN CENTER

name/ID/location
[redacted]

September 26, 2014

Ned Kalin, M.D.
Chair and Hedberg Professor of Psychiatry
Department of Psychiatry
UW-Madison
6001 Research Park Boulevard
Madison, WI 53719-1176

Dear Ned:

I am writing to express my enthusiasm to serve as a consultant for your NIH R01 application titled, “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.” As you know, I’ve been involved in research using magnetic resonance imaging (MRI) for mapping and measuring the functional and structural organization of both the human and nonhuman primate brain for more than thirteen years, and I would like to offer my expertise especially on diffusion tensor imaging (DTI) methods development and analysis. You and I have collaborated on several neuroimaging experiments in both humans and nonhuman primates and I am excited to continue to do so. As the Director of name/ID/location [redacted] I will be happy to support the development and maintenance of protocols for your project as well as advise on strategies for the analyses and interpretation of diffusion tensor imaging. I look forward to working with you on this exciting project.

Best wishes,

name/ID/location
[redacted]

Professor, Medical Physics and Psychiatry

University of Wisconsin
SCHOOL OF MEDICINE
AND PUBLIC HEALTH

Department of Psychiatry

September 30, 2014

Department of Psychiatry
University of Wisconsin
6001 Research Park
Madison, WI 53219

Dear Dr. Kalin,

I am enthusiastic in my support of your R01 application entitled “Extreme anxiety in females: the roles of the bed nucleus of the stria terminalis (BST) during the transition to adolescence in human and nonhuman primates” and I am excited to be a consultant on this project.

As you know, I have considerable experience in neuroimaging, particularly fMRI and the estimation of functional connectivity from fMRI data. I have worked extensively with your group in the past, providing support for functional MRI and functional connectivity acquisition and analysis techniques for both human and nonhuman primate datasets, and I am happy to continue doing so for the proposed project. Your proposed research to investigate the brain correlates of extreme anxiety across species and with maturation is extremely innovative and I am happy to contribute my expertise to this research.

I look forward to working with you on this project and to our continuing collaboration.

Best regards,

name/ID/location
[redacted]

Assistant Professor
Department of Psychiatry
University of Wisconsin - Madison

DEPARTMENT OF BIOMEDICAL ENGINEERING
University of Wisconsin

To: Ned Kalin, MD

Professor and Chair, Department of Psychiatry, University of Wisconsin - Madison

Dear Ned,

I am happy to provide this letter of support describing my enthusiasm and commitment regarding your NIH RO1 proposal to study the role of the bed nucleus of the stria terminals (BST) in anxiety. My lab has enjoyed the opportunity to guide and monitor convection-enhanced delivery (CED) of therapeutic agents in real-time during fifteen of your preclinical experiments in NHP models to date. My laboratory's work in studying how to precisely execute CED in the brain has demonstrated the value of real-time imaging. The heterogeneity of the brain, brain shift after gaining access to the brain, and variability in flow patterns and backflow along catheters inserted into the brain are all phenomena that drives the need for real-time MRI guidance over blinded, stereotactic approaches. This is even more so in your NHP studies relative to human studies, as the NHP brain structures are much smaller and your infusate volumes, sometimes less than 10 μl per injection, are much smaller than used in human CED applications. My laboratory was able to work effectively with your staff during our refinement studies to modify several processes to meet the tighter performance criteria needed in your NHP experiments.

My lab utilizes a unique portal to MRI scanners that allows us to gain control of the scanner hardware and customize scanning, image reconstruction, visualization, and workflows specific to the needs of MR-guided CED procedures. Rather than shoehorning commercial diagnostic procedures for interventional procedures, we have developed real-time imaging and visualization capabilities specific to CED guidance and monitoring. Instead of iterating between imaging and surgical manipulation, our approach allows concurrent real-time imaging to intuitively guide surgical manipulation of devices and drug delivery. Given the complexity and effort needed to design and execute these studies, the ability to definitively image the actual treatment region has great value. Though we have provided you qualitative imaging of the therapeutic agents to date, we also have capabilities to provide 3D, quantitative drug delivery maps.

We will continue to provide you with these guidance and monitoring capabilities in the proposed ten CED treatments. As in the past, we will provide you with an engineer throughout your surgical procedures to assist in guidance, monitoring, or other problems or questions that may occur during the procedure. We look forward to doing what we can to minimize variance in drug delivery and maximize the chances for your project's success.

Sincerely,

name/ID/location

University of Wisconsin
SCHOOL OF MEDICINE
AND PUBLIC HEALTH

Department of Psychiatry

September 29, 2014

Ned H. Kalin, M.D.
Department of Psychiatry
School of Medicine and Public Health
University of Wisconsin–Madison
6001 Research Park Blvd.
Madison, WI 53719

Dear Ned,

I am writing to confirm my enthusiasm for participating as a consultant for your proposed project, "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." I am eager to work with you on this study, as you know from our regular conversations about our common interests in stress response systems and the development of anxiety disorders.

As we demonstrated in our paper published in the *American Journal of Psychiatry* (Essex, Klein, Slattery, Goldsmith and Kalin, 2010; 167:40–46), female sex and early behavioral inhibition are predictive of chronically elevated inhibition and greater risk for anxiety disorders by adolescence. The proposed work is timely and exciting, as it promises to extend ongoing work in your lab and mine to provide much-needed information about the mechanisms that underlie the development of anxiety in girls during the transition to adolescence.

In the proposed project, I look forward to applying my extensive expertise following the longitudinal Wisconsin Study of Families and Work to the proposed project, including study design, participant recruitment and retention, analysis of longitudinal datasets, and administration and analysis of the Trier Social Stress Test.

I am very happy to continue our collaborations in this area and am very much looking forward to working with you on this important study.

Sincerely,

name/ID/location

Department of Psychiatry
School of Medicine and Public Health
University of Wisconsin–Madison

University of Wisconsin-Madison

Department of Educational Psychology

name/ID/location

September 30, 2014

Dear Dr. Kalin,

I am happy to serve as a statistical consultant for your project "Extreme anxiety in females: the roles of the bed nucleus of the stria terminalis (BST) during the transition to adolescence in human and nonhuman primates." As you know, my research focuses on developing and applying statistical methods to address practical questions in the behavioral and brain sciences. Over the last 12 years, I have served as the lead statistician on many research projects that are funded by various agencies including National Institutes of Health, Institute of Education Sciences, National Science Foundation, and Agency for Healthcare Research and Quality.

I have particular expertise in multilevel models and latent variable models, including methods for modeling change, individual differences, and human development using longitudinal data. These methods are an ideal fit for the longitudinal neuroimaging and behavioral data collected here in highly anxious girls, and I will work with your group to supervise and advise on the generation of latent growth curve models to analyze the data collected in this proposal. I will also consult with you and your team on the interpretation of results.

I look forward to working with you on this exciting and important project.

Sincerely,

name/ID/location

University of Wisconsin-Madison

Keck School of Medicine of USC

DEPARTMENT OF PSYCHIATRY AND THE BEHAVIORAL SCIENCES

Ned H. Kalin, MD
Hedberg Professor and Chair of Psychiatry
Director, Health Emotions Research Institute
University of Wisconsin School of Medicine
name/ID/location

October 1, 2014

Dear Ned,

I am writing to confirm my strongest enthusiasm to collaborate with you on your project entitled "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".

name/ID/location and the Associate Chair for California. My research focuses on identifying human disease genes for a number of psychiatric and cardio-pulmonary illnesses, as well as a basic neuroscience infrastructure project that is describing the transcriptome of the human brain throughout the lifespan. At the current time, nearly all of these projects are being approached by utilizing next generation genome and transcriptome sequencing techniques. We purchased our first Illumina Genome Analyzer in the summer of 2007 and my lab now owns two HiSeq2000s and one HiSeq2500 DNA sequencers. For humans, we have already sequenced greater than 150 whole genomes at 32X and more than 1,000 libraries for RNA-Seq, a large portion of which were part of the BrainSpan project (BrainSpan.org). We're also currently funded by the NIH Common Fund to perform single-cell RNA-Seq on thousands of cells.

As described in the preliminary data section of your proposal, this expertise has now been applied to the Rhesus monkey with the RNASeq of 48 amygdala samples. As part of this effort, we have developed the a bioinformatic pipeline for analyzing Rhesus RNA-Seq data, and version 1.0 is now available to the general scientific community (Wang et al., 2011, Bioinformatics). We plan to continue the development of this software, which is currently being supported by U01HG006531 from NHGRI, throughout your proposed project period.

I find your proposal using RNA sequencing to examine transcriptome-wide gene expression within the BST of nonhuman primates, a central region for sustained anxiety, to be extremely exciting. It is an excellent complement and extension of the research that we are doing in both the genetics of the depression and the anxiety disorders, and human brain gene expression. Hence, I am eager to be a part of this work, as it builds on your extensive validation of the rhesus monkey model of anxious temperament, and the knowledge gained will obviously translate to a better understanding of human anxiety and depression.

We have also just published the first use of RNA-Seq for single-cell transcriptomics (Qui et al, Frontiers in Genetics), so we anticipate no problems doing RNA-Seq on LCM material (100's of cells).

University of Southern California
name/ID/location

In summary, I think I am well suited for this collaboration, and my track record as a well-established psychiatric geneticist with years of experience in large-scale collaborations speaks for itself. I have thoroughly enjoyed working with you and your team over the last year and look forward to regular interactions as our fruitful collaboration continues.

Sincerely,

name/ID/location

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

Ned Kalin, MD
University of Wisconsin Medical Center
Department of Psychiatry
Madison, WI

September 30, 2014

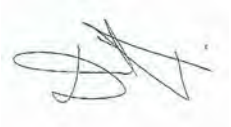
Dear Ned,

This letter expresses my ongoing commitment to work with you on your proposed project, *Extreme anxiety in females: the roles of the bed nucleus of the stria terminalis (BST) during the transition to adolescence in human and nonhuman primates*. In accordance with NIH policies, the content of letters of collaboration from intramural scientists is restricted to a description of collaborative work proposed under the grant. I understand that your project is designed to examine the neural correlates of sustained anxiety in highly anxious girls using longitudinal neuroimaging, with parallel studies in anxious female nonhuman primates to provide insight into the mechanisms and molecular underpinnings of these effects. This exciting proposal is a natural extension of your previous work on the brain basis of anxiety and depression in non-human primates, adult humans, and children. As you know, my interests have been very much along these lines, and we have worked together successfully over the last 4 years on studies of childhood anxiety disorders. My laboratory has considerable expertise in performing imaging studies in preadolescent and adolescent children with various types of psychopathology. The study that you are proposing dovetails naturally with my own work. This also is particularly relevant to my group because your work focuses on the transition from childhood to adolescence, for which there is precious little longitudinal neuroimaging data, though cross-sectional neuroimaging and epidemiological studies indicate this to be a time of increased risk for psychopathology, especially for females.

As an outgrowth of our longstanding discussions of our shared interests, our ongoing collaboration, and our recent conversations on the direction for your project, I am committed to serving as a consultant on your project. I understand that my primary roles in the proposed study will be in providing consultation for clinical diagnoses and aiding in the interpretation of neuroimaging results. Hopefully, with my input, you will be able to conceptualize your own data in relation to the most current data emerging from child psychiatry, in particular with respect to knowledge of imaging studies in pediatric anxiety. I will serve as a consultant in the ways that will best suit you, though I understand that this will likely be once in person each year, during a day-long visit, when I am happy to come to you, and monthly via conference calls.

Since I am currently employed at the Intramural Research Program of NIMH and would participate as part of my Official Duties, my group will not require any compensation. Moreover, as we have discussed, no funds from this grant can be used to support any of the work that I conduct as part of my Official Duties.

Sincerely,



Daniel Pine, M.D.
Chief, Section on Development and Affective Neuroscience
cc: Susan Amara, PhD

HARVARD UNIVERSITY
DEPARTMENT OF PSYCHOLOGY

name/ID/location [redacted]

September 22, 2014

Dear Dr. Kalin,

This letter affirms my commitment to serve as Consultant for your R01 project entitled *Extreme anxiety in females: the roles of the bed nucleus of the stria terminalis (BST) during the transition to adolescence in human and nonhuman primates*.

The proposed research incorporates an experimental fMRI task that isolates transient and sustained neural responses that track with brief and sustained affective provocation. I am the primary developer of this task (Somerville et al., 2013 *Cerebral Cortex*), and I have adapted the task for developmental populations as part of NIMH R00MH087813 (Somerville, PI). In my lab, we recently completed a fMRI large experiment focused on healthy neurodevelopment, in which we adapted the transient-sustained task for participants as young as 9 years of age. I have already shared the task with your research team and I plan to continue to consult with you and your team to ensure the task is optimized for your research objectives. I have also shared some preliminary findings with your research team to demonstrate the feasibility of applying the transient-sustained framework to developmental populations, and the initial results are promising in identifying robust reactivity of the BST (which is critical for the proposed work).

I am eager to invest my time to advise this team as they apply the task and framework to study youth varying in risk for anxiety disorders and quite frankly, I cannot wait to see the results. The proposed work (in the hands of your highly skilled and forward-thinking team) exemplifies the explanatory power of innovative translational research and I am confident that this work will make great strides toward advancing theory and mechanistic understanding of the development of anxiety dysregulation.

As Consultant, I plan to be an active contributor to the work by interacting with the research

team by email, phone, or Skype quarterly, and more often as needed. I will share the optimized developmental transient-sustained fMRI task in its entirety and help to further optimize it when the project begins. I will also plan to help solve any issues with task implementation throughout the project period. In the later stages of the project, I will consult on analytic strategies, interpretation of the results, and the broader theoretical significance of the work.

Best of luck on this extremely exciting and innovative proposal,

The Waisman Laboratory for Brain Imaging & Behavior

[redacted]

http://brainimaging.waisman.wisc.edu

Ned Kalin, M.D.
Chair, Department of Psychiatry

October 3, 2014

Dear Ned,

I'm writing to express my enthusiastic commitment to serve as a Consultant for your proposal entitled "Extreme anxiety in females: the role of the bed nucleus of the stria terminalis (BST) during the transition to adolesc nce in human and nonhuman primates." As you know, I've been involved in research using fMRI, other imaging methods, and assessments of peripheral physiological measures of affects for more than 15 year, and I have a longstanding interest in the development of affect-related psychopathology in both adults and children. I am also happy to facilitate recruitment of study participants through schools in the Madison metropolitan area. The proposed research you have described is timely and important and I look forward to working with on this project. I will provide regular, frequent consultation on all aspects of experimental design and image analysis. I will also provide access to any staff members in my lab with relevant expertise to help facilitate this work.

I look forward to working with you on this new and exciting project.

Best wishes,

[redacted]

Resource Sharing Plan

Data overview: The overarching aim of the proposal is to understand the neural correlates of sustained anxiety in anxious girls and female monkeys during the transition to adolescence, an essential step to developing more efficacious interventions for childhood anxiety and depression. During the award period, we will collect a unique data set of longitudinal multimodal neuroimaging data from anxious girls as they transition from childhood to adolescence (n=170), monkeys with excitotoxic lesions of the BST (n=10), and RNAseq data on BST neurons from female monkeys in our phenotyped brain bank (n=24). Complete datasets from anxious girls will include multimodal imaging (T1, resting fMRI, task MRI, DWI), behavioral and hormonal measures of anxiety and clinical rating scales. Complete datasets from lesion and control animals (n=20 total) will include behavioral and endocrine measures, multimodal imaging (T1, EPI [resting fMRI], FDG-PET, DWI). Data from brain-banked animals will include RNAseq analyses of central nucleus of the BST neurons In the remainder of this section, we use the term “summary-level data” to refer to data that has been anonymized, processed, analyzed, and stored in an appropriate digital format (e.g., spreadsheets, whole-brain imaging maps for nonhuman primates, or transcript alignment files).

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. 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.