PI: **Higley, Michael James**	Title: Bridging cellular and systems neuroscience: synaptic dynamics underlying behavior	
Received: 09/11/2020	Opportunity: RFA-RM-20-011	Council: 05/2021
Competition ID: FORMS-F	FOA Title: NIH Directors Pioneer Award Program (DP1 Clinical Trial Optional)	
1DP1EY033975-01	Dual: RM,OD	Accession Number: 4492387
IPF: 9420201	Organization: YALE UNIVERSITY	
Former Number: 1DP1OD031319-01	Department: MEDNSC MNBIO Neuroscience Depa	
IRG/SRG: ZRG1 BCMB-N (50)R	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 3,500,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Michael Higley	Yale University	PD/PI

Reference Letters

[redacted]

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number
7 NS; 6 MCB

2. DATE SUBMITTED
2020-09-11

Application Identifier
21-002089

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: Yale University
Department: MEDNSC MNBIO Neuroscience Depa
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: Mrs First Name*: Adrienne Middle Name: L Last Name*: Marable Suffix:
Position/Title:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* O: Private 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/DHHS

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Bridging cellular and systems neuroscience: synaptic dynamics underlying behavior

12. PROPOSED PROJECT
Start Date* Ending Date*
09/30/2021 07/31/2026

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CT-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Michael Middle Name: J Last Name*: Higley Suffix:

Position/Title: Associate Professor, Neuroscience

Organization Name*: Yale University

Department: [redacted]

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,500,000.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,500,000.00
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: Mrs First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title*: Proposal Mgr

Organization Name*: Yale University

Department: [redacted]

Division:

Street1*: [redacted]

Street2: [redacted]

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

Signature of Authorized Representative* [redacted]

Date Signed* [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover Letter.pdf

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

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

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: Yale University
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: CT-003

Additional Location(s) File Name:

Funding Opportunity Number: RFA-RM-20-011. Received Date: 2020-09-11T11:06:42.000-04:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

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

Funding Opportunity Number: RFA-RM-20-011. Received Date: 2020-09-11T11:06:42.000-04:00

SUMMARY

A major challenge to our progress in understanding the functional organization of the nervous system is the practical schism between cellular/molecular and systems sub-fields within the broader neuroscience community. For example, synaptic transmission is the fundamental mechanism by which activity propagates between neurons. While we have a detailed understanding of the cellular and molecular mechanisms underlying this process, the dynamic range and operating regime of synapses in the intact, behaving animal is essentially unknown. Based on recent data from our laboratory, our overall goal in this proposal is to investigate the hypothesis that variations in behavior over multiple time scales are associated with fluctuations in the strength of synaptic transmission within neuronal networks of the mammalian neocortex. With a groundbreaking combination of conceptual and methodological innovations, we will specifically identify the modifications of synaptic function that correspond to changes in behavioral state and perceptual learning. Specifically, we propose to monitor variation in synaptic release probability, potency, and integration for targeted circuits within the mouse visual cortex, relating these properties to behavioral state transitions and enhanced perceptual ability associated with visuomotor learning. Overall, this ambitious paradigm will generate critical new insights into the relationships between synapses, circuits, and behavior and open up new avenues of exploration that unite diverse areas of the neuroscience community.

NARRATIVE

Synaptic connections between neurons form the basis of network connectivity in the brain, and synaptic plasticity is widely believed to underlie adaptive behavior and learning. However, the dynamic range and modulation of synaptic strength *in vivo* is essentially unknown, raising a major challenge to our understanding of nervous system function. Here, we combine conceptual and methodological innovations to drive a pioneering exploration of the relationship between synaptic transmission and ongoing behavior.

FACILITIES AND OTHER RESOURCES

Laboratory: The Higley laboratory comprises ~1500 square feet in the Boyer Center for Molecular Medicine Building at the Yale School of Medicine, including a dedicated 2-photon microscopy suite (including three separate imaging systems), one electrophysiology room, one room for wet-lab space, a dedicated surgical suite, and space in a shared room for histological analysis (including fixed tissue sectioning and staining). The lab also has a dedicated confocal microscope (Zeiss 900) for histological analyses. The lab shares (with two other labs) a cell culture suite with two biosafety cabinets, centrifuge, and inverted microscope, as well as a cold room.

Environment: Yale promotes a highly collaborative environment for scientific interactions, and the PI has extensive communication and interaction with senior colleagues, including [redacted]. In addition, as a member of the Program in Cellular Neuroscience, Neurodegeneration, and Repair (CNNR), the PI participates in a monthly internal seminar series to present and discuss recent findings with colleagues.

Computer: The lab has multiple computers for data acquisition, as well as individual computers for each lab member for data analysis. We have a 350 TB data storage server dedicated to high volume imaging data. All servers are also backed up nightly to the Yale cloud storage center. In addition, the lab has full access to the Yale High Performance Computing facilities, providing cluster-based servers for analysis.

Animals: The Higley laboratory has full access to the Yale animal facility services, including animal housing, technician support, veterinary assistance, and oversight. Mice are housed in a clean Yale School of Medicine facility in the same building as the main laboratory. All facilities are AALAC approved.

Office: The PI has an office adjacent to the main laboratory. Individual lab members, including graduate students, postdoctoral fellows, and technicians, each have desks in the main laboratory.

Other:
The PI also has access to:

1. The Yale Kavli Institute for Neuroscience Technology Core. This facility, run by two experienced engineers and software designers, provides heavily subsidized design, construction, and implementation of custom equipment, including microscope systems, behavioral setups, and electronics.

2. The CNNR Core Imaging facility, including two spinning disc confocal microscopes, a TIRF microscope, and a new Zeiss 710 point-scanning confocal microscope purchased in consultation with the PI. We have full access to this equipment free of charge as a CNNR primary member.

3. Three shared conference rooms with full AV support for lab meetings and presentations.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Michael Middle Name J Last Name*: Higley Suffix:

Position/Title*: Associate Professor, Neuroscience
Organization Name*: Yale University
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: MD, PhD Degree Year: 2007

Attach Biographical Sketch*: File Name: Higley_Biosketch.pdf

Attach Current & Pending Support: File Name: Higley_DP1_OS.pdf

Funding Opportunity Number: RFA-RM-20-011 . Received Date: 2020-09-11T11:06:42.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Michael J. Higley

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Associate Professor of Neuroscience

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University	BA	05/1998	Behavioral Neuroscience
University of Pennsylvania	MD, PhD	05/2007	Neuroscience advisor: Diego Contreras
Harvard Medical School	Postdoctoral	03/2010	Neurobiology advisor: Bernardo Sabatini

A. Personal Statement

Our laboratory examines the structure and function of synapses in the mammalian neocortex and their contribution to complex circuit activity and behavior. We are particularly interested in applying an array of methodological approaches, including electrophysiology, 2-photon imaging and transmitter photo-uncaging, optogenetics, and viral tracing to both reduced preparations and intact behaving animals. In this way, we hope to bridge the gaps between molecular, cellular, and systems neuroscience.

Development, function, and plasticity of inhibitory GABAergic circuits.

A major challenge to understanding the role of GABAergic inhibition is the diversity of interneurons. In our studies, we use acute brain slice preparations and in vivo imaging to dissect the organization of GABAergic circuits and their ability to regulate cellular activity, perception, and learning. We have focused particularly on inhibition targeting pyramidal neuron dendrites, which influences both electrical and biochemical signaling in the postsynaptic cell. (Chiu et al., Science, 2013; Chiu et al., Neuron, 2018)

Cortical microcircuits underlying visually-guided behavior.

Visual information is encoded by neuronal activity in the primary visual cortex, whose diverse anatomical projections route these signals to various downstream locations. We are using novel viral tracing approaches to understand the organization of these outputs and applying novel 2-photon and mesoscopic imaging strategies to investigate the function of V1 networks during the performance of visually-guided behaviors. (Barson et al., Nature Methods, 2020; Tang and Higley, Neuron, 2020)

Neuromodulation: providing functional flexibility to cortical circuits.

Adaptive behavior requires a nervous system with sufficiently stable wiring to support long-term memory but plastic enough to adjust to rapid changes in environmental context. Much of this dynamic flexibility is provided by neuromodulators such as norepinephrine and acetylcholine. We are using a combination of approaches to study the cellular mechanisms and functional actions of neuromodulation on identified microcircuits in the mouse visual cortex (Lur and Higley, Cell Reports, 2015; Lur et al., PlosOne, 2019).

B. Positions and Honors

Positions and Employment

2010-2016 Assistant Professor, Department of Neurobiology, Yale University School of Medicine
2010- Primary Faculty, Program in Cellular Neuroscience, Neurodegeneration, and Repair
2010- Member, Swartz Program for Theoretical Neurobiology, Yale University
2012- Member, Kavli Institute for Neuroscience, Yale University
2016-2020 Associate Professor, Dept. of Neuroscience, Yale University School of Medicine
2020- Associate Professor with Tenure, Dept.of Neuroscience, Yale University School of Medicine

Other Experience and Professional Memberships

1996- Member, Society for Neuroscience
2015- Reviewing Editor, Frontiers in Neural Circuits
2016- Instructor, Neurobiology Course, Marine Biological Laboratory

Honors

1996 Howard Hughes Undergraduate Research Fellow
2002 Penn Prize for Teaching by Graduate Students
2004 William A. Jeffers Prize for Meritorious Lab Research in the Field of Neurology
2006 Saul Winegrad Award for Outstanding Dissertation in the Biomedical Sciences
2006 Louis B. Flexner Award for Outstanding Dissertation Research in the Neurosciences
2008 Kirschstein Individual NRSA Research Fellowship
2010 Smith Family Award for Excellence in Biomedical Research
2011 Alfred P. Sloan Research Fellowship
2011 Klingenstein Fellowship Award in the Neurosciences
2012 Brain and Behavior Research Foundation (NARSAD) Young Investigator Award
2013 Basil O'Connor Starter Scholar Research Award (March of Dimes)
2015 Brain Research Foundation Fay/Frank Seed Grant
2016 Inaugural Spector Award for Neuroscience Research, Yale University

C. Contributions to Science

1. My lab has extensively explored the function of GABAergic inhibition in shaping dendritic signaling in the neocortex. We made the seminal discovery that dendritic inhibition mediated by somatostatin-expressing interneurons could be compartmentalized within single spines, effectively constraining the functional spread of inhibition. In additional work, we demonstrated that GABAergic synapses formed by SST-INs selectively exhibit long-term potentiation in response to activation of NMDA-type glutamate receptors and showed that sensory experience can drive structural and functional reorganization of inhibitory synapses in the visual cortex. Most recently, we made the surprising, paradoxical discovery that tonic GABAergic signaling facilitates dendritic calcium influx by deinactivating voltage-gated channels.

a. Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies G, **Higley MJ**. Compartmentalization of GABAergic inhibition by dendritic spines. *Science*, 340:759-62, 2013. PMC3752161.
b. Kannan M, Arnold DB, Gross GG, **Higley MJ**. Visual deprivation during the critical period enhances layer 2/3 GABAergic inhibition in mouse V1. *Journal of Neuroscience*, 36:5914-9, 2016. PMC4887562.
c. Chiu CQ, Martenson JS, Yamazaki M, Natsume R, Sakimura K, Tomita S, Tavalin SJ, **Higley MJ**. Input-specific NMDAR-dependent potentiation of dendritic GABAergic inhibition. *Neuron*, 97:368-377, 2018. PMC5777295.
d. Chiu CQ, Morse TM, Nani F, Knoflach F, Hernandez MC, Jadi M, **Higley MJ**. Tonic GABAergic activity facilitates dendritic calcium signaling and short-term plasticity. *BioRXiv*

2. I have also investigated the mechanisms and consequences of neuromodulation in cortical circuits. Using combinations of ex vivo electrophysiology, 2-photon imaging and glutamate uncaging, and optogenetics, we showed that acetylcholine and dopamine modulate synaptic transmission by distinct pre- and postsynaptic mechanisms. We then found that adrenergic and GABAergic signaling drives modification of excitatory

synapses by divergent PKA-dependent pathways. We also showed that the antidepressant ketamine drives persistent disruption of synaptic modulation in the cortex.

a. **Higley MJ**, Soler-Llavina GJ, Sabatini BL. Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nature Neuroscience*, 12:1121-1128, 2009. PMC2733934.

b. **Higley MJ**, Sabatini BL. Competitive regulation of synaptic calcium influx by D2 dopamine and A2A adenosine receptors. *Nature Neuroscience*, 13:958-66, 2010. PMC2910780.
c. Lur G, **Higley MJ**. Glutamate receptor modulation is restricted to synaptic microdomains. *Cell Reports*, 12:326-34, 2015. PMC4920052.
d. Lur G, Fariborzi M, **Higley MJ**. Ketamine disrupts neuromodulatory control of glutamatergic synaptic transmission. *PLoSOne*, 14:e0213721, 2019. PMC6415832.

3. We have also applied 2-photon and widefield "mesoscopic" imaging to investigating the neuronal and synaptic dynamics of visual microcircuits in the awake behaving mouse. These studies include the first characterization of visual response properties in projection-specific layer 5 neurons. More recently, we demonstrated that corticopontine cells selectively encode and are necessary for visual perception in a conditioned eyeblink task. To further investigate functional architecture of cortical networks, we have developed a novel imaging strategy using simultaneous 2-photon and widefield imaging to link individual neurons to large-scale circuits.

a. Lur G, Vinck MA, Tang L, Cardin JA, and **Higley MJ**. Projection-specific visual feature encoding by layer 5 cortical subnetworks. *Cell Reports*, 14:2538-45, 2016. PMC4805451.
b. Barson D, Hamodi AS, Cardin JA, Crair MC, **Higley MJ**. Simultaneous mesoscopic and 2-photon imaging of neuronal activity in cortical circuits. *Nature Methods*, 17:107-113, 2020. PMC6946863.
c. Tang L, **Higley MJ**. Layer 5 circuits in V1 differentially control visuomotor behavior. *Neuron*, 105:346-54, 2020. PMC6981039.
d. Puscian A, Benisty H, **Higley MJ**. NMDAR-dependent emergence of behavioral representation in primary visual cortex. *Cell Reports,* 32:107970, 2020. PMC7431963.

4. The combination of 2-photon imaging, transmitter uncaging, and calcium-sensitive fluorescence indicators has driven fundamental breakthroughs in our understanding of synaptic and network function. We have carried out multiple studies aimed at developing new tools for manipulating synaptic function and novel approaches for interpreting calcium imaging data.

a. **Higley MJ** and Sabatini BL. Calcium signaling in dendrites and spines: practical and functional considerations. *Neuron*, 59:902-13, 2008.
b. **Higley MJ** and Sabatini BL. Calcium signaling in dendritic spines. *Cold Spring Harbor Perspectives in Biology,* 4:a005686, 2012. PMC3312680.
c. Olson JP, Kwon HB, Takasaki KT, Chiu CQ, **Higley MJ**, Sabatini BL, Ellis-Davies GC. Optically selective two-photon uncaging of glutamate at 900nm. *Journal of the American Chemical Society*, 135:5954-7, 2013. PMC4079120.
d. Amatrudo JM, Olson JP, Lur G, Chiu CQ, **Higley MJ**, Ellis-Davies GC. Wavelength-selective one- and two-photon uncaging of GABA. *ACS Chemical Neuroscience*, 5:64-70, 2014. PMC3894722.

5. Synaptic dysfunction is hypothesized to play a major contributory role to a number of neuropsychiatric disorders, including autism and schizophrenia. We have leveraged our expertise in both synaptic biology and neuromodulatory signaling to investigate the relationships between altered synaptic function, brain circuit organization, and behavior.

a. Picciotto MR, **Higley MJ**, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron*, 76:116-29, 2012. PMC3466476.
b. Einstein EB, Asaka Y, Yeckel MF, **Higley MJ**, Picciotto MR. Galanin-induced decreases in nucleus accumbens/striatum excitatory postsynaptic potentials and morphine conditioned place preference require both galanin receptor 1 and galanin receptor 2. *European Journal of Neuroscience*, 37:1541-9, 2013. PMC3648588.

c. Gamo NJ, Lur G, **Higley MJ**, Wang M, Paspalas CD, Vijayraghavan S, Yang Y, Ramos BP, Peng K, Kata A, Boven L, Lin F, Roman L, Lee D, Arnsten AF. Stress impairs prefrontal cortical function via D1 dopamine recotor interactions with hypoerpolarization-activated cyclic nucleotide-gated channels. *Biological Psychiatry*, 78:860-70, 2015. PMC4524795.
d. Batista-Brito R, Vinck M, Ferguson KA, Chang JT, Laubender D, Lur G, Mossner JM, Hernandez VG, Ramakrishnan C, Deisseroth K, **Higley MJ**, Cardin JA. Developmental dysfunction of VIP interneurons impairs cortical circuits. *Neuron*, 95:884-895, 2017. PMC5595250.

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/pubmed?term=Higley%2C%20Michael%20J%5BFull%20Author%20Name%5D&cmd=DetailsSearch

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 MH099045-08 Higley (PI) 09/20/17-10/31/22
NIH/NIMH
"Cellular mechanisms of GABAergic inhibition in neocortical dendrites"
This grant is to study the basic mechanisms and functions of GABAergic synapses targeting the dendrites of neocortical pyramidal cells.
Role: PI

R21 MH121841-01 Higley (PI) 12/01/19-11/30/21
NIH/NIMH
"Behavior-dependent classification of neocortical cell types"
This grant is for the development of novel activity-dependent labeling and classification of neocortical neurons based on variable response to behavioral state.
Role: PI

R01 MH113852-04 Jessica A. Cardin and Michael J. Higley (co-PIs) 08/01/17-05/31/22
NIH/NIMH
"Role of GABAergic interneurons in developmental dysregulation of cortical function"
This grant is to study the role of MeCP2 expression in cortical interneurons in the development and function of cortical circuits.
Role: PI

SFARI Research Grant Jessica A. Cardin and Michael J. Higley (co-PIs) 10/01/18-09/30/21
Simons Foundation
This grant is to combine novel in vivo imaging approaches and CRISPR/Cas9 gene editing to investigate convergent network phenotypes in diverse models of autism.
Role: PI

R01 NS105640-03 Tony Koleske and Michael J. Higley (co-PIs) 04/01/18-01/31/23
NIH/NINDS
"Impact of excitatory synapse maturation on synaptic plasticity and stability"
This grant is to explore the molecular mechanisms underlying excitatory synaptic maturations and function.
Role: PI

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

2. *Program Income Section

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

○ Yes ● No

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

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

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

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

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

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

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ❍ No

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

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

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

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

Tracking Number: GRANT13203404
Funding Opportunity Number: RFA-RM-20-011. Received Date: 2020-09-11T11:06:42.000-04:00

ESSAY

1. SCIENTIFIC AREA: Primary: 7 NS (Neuroscience); Secondary: 6 MCB (Molecular and Cellular Biology)

2. PROJECT TITLE: Bridging cellular and systems neuroscience: synaptic dynamics underlying behavior

3. PROJECT DESCRIPTION:

The scientific challenge

Neuronal networks are formed by synaptically coupled cells whose coordinated activity underlies cognition and the generation of behavior. Network connectivity must exhibit long-term stability to promote storage of learned skills and memories. However, flexibility is also necessary for the acquisition of new knowledge and the dynamic allocation of resources in response to changing goals and unfamiliar environments. The cellular mechanisms that support a balance between functional rigidity and plasticity are not well understood, particularly in the context of the behaving organism. Moreover, the long-standing fragmentation of the neuroscience community into cellular/molecular and systems subfields raises substantial barriers to progress in this area. Based on recent data from our laboratory, **our overall goal in this proposal is to investigate the hypothesis that variations in behavior over multiple time scales are associated with fluctuations in the strength of synaptic transmission within neuronal networks of the mammalian neocortex**. With a groundbreaking combination of conceptual and methodological innovations, we will specifically identify the modifications of synaptic function that correspond to changes in behavioral state and perceptual learning.

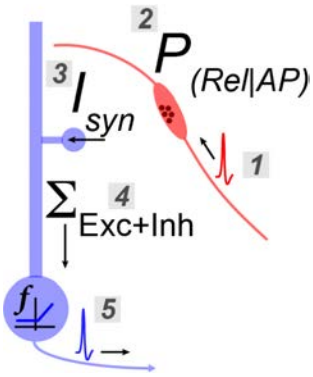

Figure 1. Steps in synaptic transmission: Presynaptic action potentials (1) induce probabilistic transmitter release (2), resulting in postsynaptic currents through activated receptors (3) that are integrated along the somatodendritic arbor (4), influencing the nonlinear generation of action potentials (5). Diverse forms of plasticity can modulate each step.

Synaptic signaling and plasticity has long been a focus of neuroscientific research. Generally, synaptic transmission occurs in a concerted process (***Figure 1***): 1) neuronal actional potentials propagate along presynaptic axons, 2) depolarization of the presynaptic bouton induces calcium-dependent neurotransmitter vesicle fusion, 3) transmitter molecules diffuse across the synaptic cleft and bind to receptors that induce current flow in the postsynaptic cell, 4) changes in postsynaptic membrane potential associated with multiple inputs are integrated, producing voltage fluctuations at the perisomatic spike-initiation zone, and 5) suprathreshold depolarization induces the generation of action potentials. **Each of these steps can exhibit diverse forms of activity-dependent short- and long-term plasticity that are broadly assumed to underlie behavioral flexibility and learning**. However, most knowledge of these processes derives from *ex vivo* preparations where physiological activity patterns and biochemical signaling regimes may deviate substantially from *in vivo* conditions (Destexhe et al., 2003), leaving the dynamic range of synaptic function and plasticity mechanisms during behavior essentially unknown.

A growing literature demonstrates the capacity for network flexibility associated with changes in behavior. For example, classification of behavioral states by levels of arousal (e.g., variation in pupil diameter) or motor activity (e.g., locomotion) reveals systematic modulation of neuronal firing rates and the gain of responsiveness to sensory inputs (Vinck et al., 2015). We recently combined 2-photon imaging and behavioral analyses to show that pyramidal neurons in mouse primary visual cortex exhibit significant enhancement in their visual responses during arousal that corresponds to improved perceptual ability (Tang and Higley, 2020)(**Figure 2A-D**). A variety of mechanisms have been proposed for these dynamics, including a role for neuromodulators such as acetylcholine and norepinephrine that we have previously shown can directly modify synaptic transmission (Higley et al., 2009; Lur and Higley, 2015)(**Figure 2E-F**). Cortical activity is also influenced by learning, such as the enhancement of selectivity for visual stimulus features associated with reward (Poort et al., 2015). We found that the association of a visual cue with an aversive

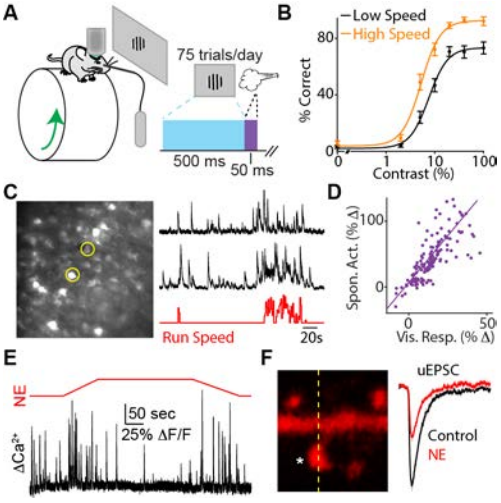

Figure 2. A, Setup for visual task acquisition. Mice are trained to associate a small stimulus with an aversive air-puff. B, Running speed correlates with task performance. C,D, 2-photon calcium imaging reveals locomotion enhances spontaneous and visually evoked activity. E, Intracortical infusion of norepinephrine (NE) suppresses cellular activity *in vivo*. F, Application of NE suppresses glutamate uncaging-evoked synaptic responses *ex vivo*. Adapted from Lur and Higley 2015; Tang and Higley 2020.

stimulus produced significant intracortical plasticity, reducing visual responses and driving the emergence of accurate behavioral representations in cortical networks that required cell-autonomous expression of NMDARs (Puścian et al., 2020)(**Figure 3**).

We propose that these forms of functional plasticity are dependent on changes in the strength of synaptic transmission within visual cortex over short and long timescales. However, the major challenge to investigating this hypothesis is the necessity for simultaneous monitoring of multiple signals across distinct subcellular compartments in the awake, behaving animal. My training and experience bridging synaptic and systems neuroscience, along with my success in developing novel methodological strategies for studying neuronal function, places me in a unique position to make fundamental breakthroughs in understanding the links between synaptic function, brain activity, and behavior.

The importance of the problem

Synaptic transmission is the fundamental mechanism by which activity propagates between cells in the nervous system. While we have a detailed understanding of the cellular and molecular mechanisms underlying this process, the dynamic range and operating regime of synapses in the intact, behaving animal is essentially unknown. This extraordinary gap in knowledge remains a critical barrier to progress in the broader neuroscience community. For example, many genes linked to neurodevelopmental disorders such as autism and schizophrenia code for proteins either directly or indirectly involved in synaptic transmission. Indeed, the "balance" of synaptic excitation and inhibition is hypothesized to be a critical parameter in normal brain function, despite limited data on how these signals operate *in vivo*. The development of a platform for understanding synaptic dynamics *in vivo* and their dysregulation by disease-linked mutations is critical for forming mechanistic links between genes and behavior. For a second example, computational neural networks have become an essential component of efforts to model brain function, both for biological and artificial intelligence goals. While such networks offer powerful approaches to understand the organization of neuronal circuits, most versions do not incorporate synaptic dynamics as a variable parameter, potentially omitting a critical mechanism for tuning network function. Real-world data on how synapses function in the intact, behaving animal are critical for designing and testing the next generation of strategies in computational neuroscience. Overall, understanding the operating regime and regulatory mechanisms of synaptic transmission in the intact, behaving animal is fundamental to synergizing the strengths and efforts of cellular and systems neuroscience disciplines.

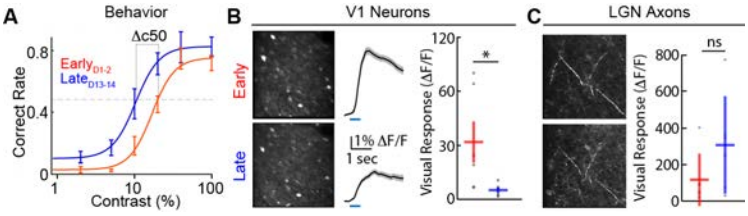

Figure 3. A, Visual conditioning drives perceptual learning, seen as a left-shift in the perceptual threshold (c50) from early to late stages of training. B, 2-photon imaging of visual cortex neurons reveals suppression of responses during training. C, Imaging thalamic axons reveals that ascending input is not altered during training. Cortical plasticity requires cell-autonomous NMDAR expression (not shown). Adapted from Puscian et al., 2020.

Pioneering approaches that will drive groundbreaking results

Light-based approaches for monitoring and manipulating electrical and biochemical signaling in targeted subcellular compartments provide an opportunity to investigate synaptic function at an unprecedented level of detail. My lab has an established track record for developing and applying novel optical methods in combination

with molecular and behavioral strategies to drive discoveries of synaptic and circuit organization in the mouse neocortex (Chiu et al., 2013, 2018; Puścian et al., 2020; Tang and Higley, 2020). For example, we recently developed a novel system for simultaneous 2-photon and wide-field "mesoscopic" imaging, given us insight into the functional connectivity of single cells within the global cortical network (Barson et al., 2020)(**Figure 4A-B**). We have expanded this approach to include multi-color imaging in both modalities, allowing us to monitor multiple coincident cellular and neuromodulatory signals in the awake, behaving mouse (**Figure 4C-D**).

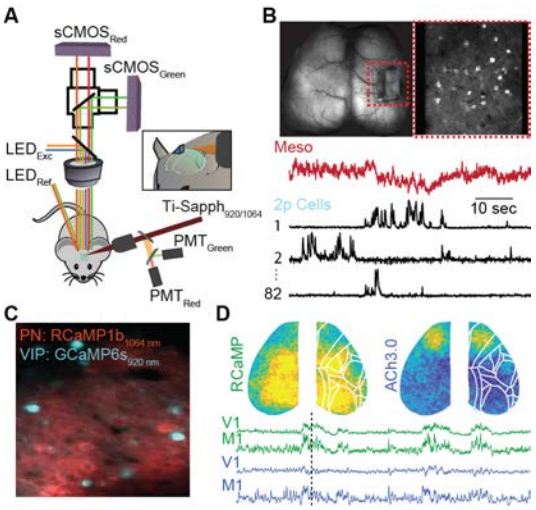

Figure 4. A, Custom-built system for simultaneous dual-color 2-photon and wide-field "mesoscopic" imaging in the awake mouse. B, Examples showing whole-cortex mesoscopic and local 2-photon calcium imaging. C, Example 2-color imaging of RCaMP1b-expressing pyramidal neurons (PN) and GCaMP6s-expressing VIP-interneurons (VIP). D, Example 2-color mesoscopic imaging of neuronal (RCaMP) and cholinergic (ACh3.0) activity. Adapted from Barson, Hamodi, et al., 2020.

Here, we propose to apply these methods to studying synaptic signaling and plasticity in the awake, behaving animal. We will address the general question: *What factors regulate the dynamic range of synaptic strength in the intact neocortex?* The paradigm described below is not meant as a detailed set of experimental plans, but rather an outline of general approaches that will be able to adapt to unexpected directions as they pioneer new fields of inquiry.

Synaptic strength depends on a combination of release probability and potency (response magnitude given a successful release event), followed by integration that is shaped by postsynaptic excitability. Investigating these processes fundamentally requires the simultaneous monitoring of signals in at least two distinct subcellular compartments, a challenge met by combining multi-color imaging of diverse fluorescent reporters. All these studies will be carried out in the awake mouse using our established approaches that include detailed behavioral state monitoring (e.g., locomotion, fluctuations in pupil diameter, high-speed video capture of facial and body movements, changes in heart and respiratory rates). Imaging will also be carried out in mice learning to perform visually-guided tasks that we have found to drive plasticity of both perceptual ability and neuronal activity within visual cortex (Puścian et al., 2020; Tang and Higley, 2020). In this way, all measures of synaptic function will be directly related to ongoing behavioral variables.

In the mammalian neocortex, excitatory synapses have been described as either high-probability (e.g., thalamocortical) or low-probability (e.g., corticocortical), potentially mapping on to feed-forward versus feed-back information flow (Sherman, 2016). To measure release probability *in vivo*, we will pair simultaneous 2-photon imaging of a red-fluorescent calcium indicator such as jRCaMP (reporting presynaptic action potentials) and a green-fluorescent glutamate sensory such as iGluSnFr (reporting a successful release event) expressed in targeted axons. We will compare a variety of pathways, such as thalamocortical inputs to visual cortex from the lateral geniculate nucleus and feedback projections from higher-order visual and frontal association areas. Using a similar dual-color imaging strategy, we will monitor postsynaptic potency as the calcium signal measured in a dendritic spine corresponding to an identified glutamatergic release event. Comparing different locations along the somatodendritic axis will allow us to determine how behavior modifies synaptic inputs from distinct inputs, with feed-forward and feed-back projections targeting proximal versus distal spines, respectively. Moreover, combining imaging with 2-photon photo-release of exogenously applied caged glutamate (i.e., through a locally implanted guide cannula) or 2-photon stimulation of optogenetic proteins expressed in the presynaptic axon will allow us to evoke precisely timed synaptic activation to compare with spontaneous signals. We can also leverage our experience with simultaneous 2-photon and mesoscopic imaging (see Figure 4) to monitor network fluctuations in neuromodulatory signaling via reporters of cholinergic or adrenergic release (Jing et al., 2018). We will further expand these studies with *in vivo* application of 2-photon fluorescence lifetime imaging (2pFLIM), an approach that provides high spatial resolution monitoring of intracellular molecules including cAMP and

CaMKIIa that are strongly linked to the regulation of synaptic strength in reduced preparations (Laviv et al., 2016). Finally, all of these approaches will be combined with pharmacological and genetic perturbations to determine the causal roles of specific pathways in regulating synaptic function. Overall, this ambitious paradigm will generate critical new insights into the relationships between synapses, circuits, and behavior and open up new avenues of exploration that unite diverse areas of the neuroscience community.

4. EVIDENCE OF THE PI'S INNOVATIVENESS

My scientific career has been defined by a strong motivation to cross established boundaries of neuroscientific disciplines in order to generate a combined mechanistic and conceptual understanding for how the nervous system is functionally organized. As a graduate student, I studied the ability of single neurons to integrate sensory information *in vivo*, focusing on how inhibition influenced cellular output (Higley and Contreras, 2003, 2006). For my postdoctoral work, I made an unconventional decision in switching to an *ex vivo* model, learning to apply recently developed strategies for 2-photon imaging and transmitter uncaging to identify mechanisms of neuromodulation at single synapses (Higley and Sabatini, 2010; Higley et al., 2009). Over my career, I have generally found that synaptic biologists do not routinely consider network dynamics associated with the intact brain and systems neuroscientists do not consider synaptic dynamics. My unusual training path has given me uncommon experience and insight into these conceptually linked but often practically isolated areas of research that I hope to apply with this proposal.

In my own lab, I have continued to break conceptual and methodological barriers to our understanding of cortical organization and function. By combining electrophysiology, dual glutamate and GABA uncaging, imaging, and optogenetics, we made the seminal observation that synaptic inhibition could act with spatial precision rivaling that of excitatory inputs to sculpt dendritic integration (Chiu et al., 2013). We further showed that these dendritic inhibitory synapses undergo a unique form of activity-dependent plasticity that provided one of the first mechanistic descriptions of how excitatory and inhibitory strength is coordinated subcellularly (Chiu et al., 2018). More recently, we provided the first characterization of how distinct, anatomically defined subclasses of layer 5 pyramidal neurons are functionally connected and causally linked to visual behavior (Lur et al., 2016; Tang and Higley, 2020). For these studies, we developed a novel paradigm for studying visual perception based on the surprising finding that Pavlovian eyeblink conditioning requires visual cortex for processing sensory cues (Puścian et al., 2020; Tang and Higley, 2020).

In addition to these conceptual innovations, we have developed novel imaging strategies for linking multiple levels of analysis. We recently described a system for simultaneous 2-photon and wide-field imaging capable of monitoring single cell and global cortical dynamics in the awake, behaving mouse (Barson et al., 2020). In conjunction with this work, we developed an approach for pan-brain expression of AAV-driven constructs using neonatal injections into the cerebral vasculature. These methodological developments are at the heart of our continuing efforts to bridge our understanding of the very small and the very large elements of cortical circuits.

To complete the proposed studies, I will also continue to leverage our successful collaborations with groups spanning diverse fields of neuroscience. With the laboratory of [redacted], we have developed novel imaging and behavioral strategies for studying the mouse visual system. In collaboration with [redacted], we are implementing tools to combine calcium imaging with functional magnetic resonance imaging (fMRI), allowing us to bridge cellular and network data sets between rodent and human subjects. We also collaborate with [redacted], an applied mathematician with expertise in analyzing multi-modal data sets (such as neural activity and behavioral state variables). We are collaborating with [redacted] to develop novel molecular tools for targeted manipulation of individual synapses.

5. How the Planned Research Differs from Past and Current Work

Much like the broad field of neuroscience, our past and ongoing work focuses independently on understanding synaptic function and plasticity, or *in vivo* circuit dynamics and behavior. It is time for both our lab and the community to develop strategies for bridging these gaps. We propose to make novel and synergistic use of the methods and concepts we have successfully applied to each half of this challenge, with the ultimate goal of broadening the scope of synaptic and systems neuroscience. Specifically, our proposal to investigate the function of individual synaptic contacts in the context of the intact, behaving animal, represents a fundamental new area of exploration for our group.

6. Suitability for the Pioneer Award Program

There are five reasons why this important project should be funded through the Pioneer Program. First, examining the dynamic function of synapses in the awake, behaving animal is an ambitious departure from our ongoing research directions and unlikely to be funded through more traditional mechanisms. Second, our overall focus is substantially different from current thinking in the field, given the long-standing isolation of the synaptic and systems communities. Third, our proposal represents a significant risk for potentially high reward. Fourth, our highly interdisciplinary approach, which includes cell biology, synaptic physiology, systems neuroscience, and behavioral analyses, makes use of cutting edge combinations of methodological tools and strategies. This unique approach is also a barrier to traditional funding programs. Fifth, the personnel (including expert collaborators) and technological developments could not be funded through a modular R01 budget.

7. Confirmation of Research Effort Commitment

If this proposal is funded, I will commit a minimum of 51% of my research effort to it.

8. References

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	❍ Yes ● No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

VERTEBRATE ANIMALS

1. In order to carry out the studies outlined in this proposal, we will use adult mice. As this proposal does not include specific experimental plans, we note that all studies involve rigorous power analyses at the outset to estimate the number of animals needed for adequate statistical comparisons. All efforts are made to minimize the use of animal subjects without sacrificing the quality or interpretability of or findings.

2. We will use mice because 1) this is currently the only way to perform experiments to target specific cell types and 2) mouse cortex is a commonly used model, allowing us to easily compare our experimental results with those of others. While we make every effort to limit the number of animals used by recording from each animal for long periods, at present there is no alternative to using animal models for this work. Cortical network function cannot be studied in cell cultures or with any other more reduced preparation aimed at limiting animal use at this time.

3. All animals used in this study will be housed in the mouse facility of the Yale Medical School in the same building as the PI's laboratory. This barrier facility is operated by the Yale Animal Resources Center (YARC). YARC works in coordination with the Yale Institutional Animal Care and Use Committee (IACUC) and provides professional veterinary, surgical, and animal care services. The permanent YARC staff includes nine veterinarians, and the facility housing the animals for this study includes a staff of twelve animal care and support personnel. YARC also provides training on the care and use of animals for Yale faculty, staff, and trainees.

4. All animals used in the proposed work will be biopsied (ear) for genotyping as necessary and surgically implanted with a headpost and glass cranial window. After all surgical and biopsy procedures, animals are treated with pain medication and monitored for several subsequent days for proper intake of fluids and food and for signs of pain and infection. For surgery, anesthesia is carried out under isoflurane anesthesia in a stereotaxic apparatus, and mice are monitored continuously for breathing and heart rate. Surgery is preceded by infiltration of the scalp with subcutaneous lidocaine to reduce pain. Anesthetized mice will be maintained at 37°C by a homeothermic blanket system. Following surgical implantation, mice are allowed to recover, treated with analgesics, and returned to the home colony. Behavioral studies are carried out no sooner than one week following surgery. Mice are acclimated to head fixation for a minimum of one week before any experiments begin.

5. At the conclusion of the study, mice are euthanized with an overdose of isoflurane and perfused intracardially. This procedure is consistent with AVMA guidelines and the policies of the Yale IACUC.

BIOHAZARDS

Many experiments in this proposal utilize adenoassociated viral (AAV) vectors for delivery of transgenic material into mouse brain. At Yale University, AAV is deemed a level 1 low-risk hazard (BL1). Per University Environmental Health and Safety regulation, injections are performed using appropriate precautions including personal protective gloves and gowns. Injected mice are housed in our approved facility, and all tissue is disposed of using standard biohazard protocols.

AUTHENTICATION OF KEY BIOLOGICAL RESOURCES

1. The majority of chemical resources (e.g., solutions, pharmacological agents) used in the present application are standard laboratory reagents, purchased from well-established sources (e.g., Fisher Scientific, Sigma-Aldrich, Tocris).

2. All mouse lines are routinely genotyped to assure appropriate expression of genes of interest. All Cre lines have been validated in-house by crossing to fluorescent reporter mice and confirmed with immunohistochemistry.

3. Viruses used for expression of fluorescent reporters are (1) purchased from either Addgene or the University of North Carolina Vector Core or (2) produced in-house by our labs. Commercial vectors are authenticated and titered by the supplier and have been vetted for several years by large numbers of laboratories. In-house vectors are produced using standard AAV-production kits, authenticated by restriction digest, and titered using qPCR. In both cases, quality control is further supported by experimental observation.