PI: **FROEMKE, ROBERT CROOKS**	Title: Synaptic basis of perceptual learning in primary auditory cortex	
Received: 07/05/2016	Opportunity: PA-16-160	Council: 01/2017
Competition ID: FORMS-D	FOA Title: NIH Research Project Grant (Parent R01)	
2R01DC012557-06	Dual: MH,LM	Accession Number: 3954637
IPF: 5998304	Organization: [redacted]	
Former Number:	Department: Otolaryngology	
IRG/SRG: ZRG1 AUD-Z (90)S	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 6: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	Organization:	Role Category:
Robert Froemke	[redacted]	PD/PI

Additions for Review

Accepted Publication Froemke.pdf

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
DC012557

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: [redacted]
Department: Sponsored Programs Admin
Division:
Street1*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Irfana Middle Name: Last Name*: Ahmed Suffix:
Position/Title: Grants Specialist
Street1*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
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

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Synaptic basis of perceptual learning in primary auditory cortex

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2017 03/31/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
[redacted]

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Robert Middle Name: C Last Name*: Froemke Suffix:
Position/Title: Assistant Professor
Organization Name*: [redacted]
Department: Otolaryngology
Division: [redacted]
[redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]

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*: Anthony Middle Name: R Last Name*: Carna Suffix:
Position/Title*: Sr Dir-Sponsored Prog Adm(SPA)
Organization Name*: [redacted]
Department: Sponsored Programs Admin
Division:
Street1*: [redacted]
[redacted]
[redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*
Anthony.Carna

Date Signed*
07/05/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:coverletter_final.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: [redacted]
Duns Number: [redacted]
[redacted] [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

File Name

Additional Location(s)

Funding Opportunity Number: PA-16-160. Received Date: 2016-07-05T15:19:02.000-04:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5____6

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 06-09-2016

Animal Welfare Assurance Number A3435-01

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*	project_summary_final.pdf
8. Project Narrative*	health_relevance_final.pdf
9. Bibliography & References Cited	references_cited_final.pdf
10.Facilities & Other Resources	facilities_final.pdf
11.Equipment	equipment_final.pdf

Tracking Number: GRANT12208477
Funding Opportunity Number: PA-16-160. Received Date: 2016-07-05T15:19:02.000-04:00

Project Summary

The brain has the remarkable capability to change in response to experience. This plasticity is essential for learning and memory, and is an important feature of the auditory cortex, especially for learning the significance of sensory signals such as speech, for the use of devices such as cochlear implants, and for recovery after short-term deafness. These changes are thought to occur primarily at synapses, basic units of information processing and plasticity. Long-term synaptic plasticity requires sensory experience and activation of neuromodulatory systems which convey behavioral context to local cortical circuits. However, little is known about the interactions between synaptic inputs and release of neuromodulators in vivo, making it challenging to relate perceptual learning to plasticity in the auditory cortex or other brain areas. Recently we developed an approach to measuring dynamics of synaptic modifications for hours, coupled with imaging techniques enabling us to monitor the same cells over days during training, directly monitoring and manipulating activity in behaving mice. These approaches allow for a close examination of links between modulation, cortical plasticity and auditory perceptual learning.

Specifically, we will study how auditory perceptual training activates the cholinergic vs noradrenergic modulatory systems. These two modulators are principally involved in selective attention towards behaviorally-important stimuli, general arousal, and learning. However, there may be important functional differences in these systems in terms of when they are active during different phases of training or consequences of cholinergic and noradrenergic modulation on auditory neurons for contextual information processing. This proposal describes a series of imaging, recording, optogenetic, and behavioral experiments that will compare and contrast the effects of locus coeruleus activation and norepinephrine release vs the effects of nucleus basalis activation and cholinergic modulation on the primary auditory cortex of behaving mice. Many studies have highlighted the importance of recording in awake animals during behavior, and we will first examine how ensembles of excitatory and inhibitory neurons are affected by learning over the entire duration of training, as animals go from naïve and poor performers, to having reliable performance on an auditory detection and recognition task we have used in the lab for years. Next, we determine when and how cholinergic and noradrenergic modulation affect behavioral and neural responses. Finally, we will make some of the first direct measurements of modulatory neuron responses, asking how these systems are activated by task-relevant variables such as sounds linked to reward.

In summary, here we use in vivo recording and imaging methods to ask how behavioral training engages and modifies noradrenergic and cholinergic systems, to collectively affect auditory cortical processing and persistently improve auditory perceptual abilities in behaving mice.

Project Narrative

Neuroplasticity- the ability of the brain to change in response to experience- is an essential feature of the auditory cortex, especially for speech and language learning as well as the successful use of devices such as cochlear implants. However, it is unclear how motivational state and behavioral training drive plasticity within the central auditory system. The experiments to be performed in this proposal provide essential data on basic mechanisms of neuromodulation and plasticity in the auditory cortex, required for improvement of prosthetic design and therapeutic strategies for treatment of deafness and language disorders.

Facilities and Other Resources

Laboratory Space

Our main laboratory space is approximately 1500 square feet on the fifth floor of the [redacted] Institute of [redacted] School of Medicine. Both the [redacted] Institute and my laboratory are well equipped for electrophysiological, imaging, histological, optogenetic, and behavioral experiments. The laboratory has space for 10-15 people to have their own benches and desks. We have two large walk-in acoustic isolation chambers within the main lab space (each 8' x 6') fully equipped for in vivo extracellular and whole-cell electrophysiological experiments, and two brain slice rig for fluorescence micropscopy and quadruple whole-cell recordings also within the main lab space. In a dedicated room down the hall from the main lab, we have a 2-photon in vivo imaging system with a Mai Tai Deepsee laser (Spectra-Physics), built with a Movable Objective Microscope (Sutter) for imaging from auditory cortex in awake animals. Each rig is dedicated full-time to one student or postdoc. Each in vivo recording rig has a computer, a preamplifier, a digitizer and whole-cell recording amplifier (Molecular Devices MultiClamp), a digital signal processor for acoustic stimulus generation (TDT), a small tabletop laser or LED system for optogenetic stimulation, and a current generator for electrical stimulation (Grass). The lab is outfitted with a workbench for electronics and small parts machining, and two chemical fume hoods. I have two quiet, isolated annexes for behavioral experiments (50-100 square feet), one for operant conditioning in rats, the other for behavioral studies in mice as described in the current application. We have one multielectrode system for chronic electrophysiological recordings and stimulation in freely-moving animals (BlackRock).

I designed the lab space and renovations were complete by the time I arrived at [redacted] on 5/1/10. The main lab space is an open area, with rigs and desks along the walls, facilitating and maximizing interactions, discussions, and my ability to oversee and mentor trainees. I received additional space in 2015.

Animal Facilities

The Institute is particularly well-suited for physiological and behavioral studies in rodents. Our animals are housed in the Medical Center and supervised by a full-time veterinarian and her staff. Each animal is inspected daily, food and water provided, and bedding is changed 2/week. Vet staff are on-call and monitor implanted animals daily to ensure their health. We have authorization to perform surgeries, electrophysiological recordings, and behavioral experiments all within our own lab space, and the PI and all lab members receive annual training in the ethical care and use of animals in research. Consequently, we will carry out our studies in an environment with great technical expertise and experience.

Computers

Each desk and rig has a PC dedicated for electrophysiological data acquisition, analysis, and routine laboratory use. All computers have ethernet access to a lab printer and network services, including licensed software such as Matlab. The Skirball Institute has excellent computer support via an in-house IT support staff.

Office Space

The PI's office is approximately 120 square feet, located directly within the main laboratory space. I have a desk, a networked PC, a black-and-white printer and a color scanner/printer, and a table and extra chairs for meetings with other faculty and students.

Clinical

The PI's primary departmental appointment is within [redacted] department of otolaryngology. There is a large and active cochlear implant center at [redacted] I have participated in Grand Rounds in otolaryngology and in the Child Study Center at [redacted], and meet regularly with clinical faculty, especially those working in psychiatry, hearing loss and cochlear implants (including Tom, Susan, and Mario), and rehabilitation medicine, to discuss issues in potential therapeutic approaches, training programs, adaptation and plasticity.

Other

[redacted] Medical provides an optimal environment to conduct the proposed studies, both intellectually and in terms of the available resources. The [redacted] Institute of Biomolecular Medicine at [redacted] Medical Center consists of ~30 laboratories, and [redacted] Medical houses a large number of laboratories dedicated to neuroscience in general and cortical physiology in particular. Importantly, a new initiative- the [redacted] Neuroscience Institute- has recently

started at [redacted] School of Medicine. This new opportunity has made [redacted] an even more exciting and collaborative place to work, leading to the hiring of several new junior and senior faculty, including [redacted] as Director. We have had frequent discussions with [redacted] about our research, and have collaborated on projects involving 2-photon imaging as well as neuromodulation and inhibitory plasticity in the forebrain. Our laboratory is also on the same floor as [redacted] laboratory, so we have meetings regarding 2-photon imaging in behaving mice, cortical organization, plasticity, and rodent behavior with [redacted] his group. Likewise, we have regular discussions (and a few direct collaborations) with [redacted], [redacted], experts on cortical circuit development and organization, in vivo whole-cell recording, cortical neuromodulation, and behavioral experiments in rodents, respectively. We also have close contact with colleagues at the Center for Neural Science, located about a mile from the Medical Center, including [redacted] who we have collaborated with on electron microscopic studies of neuromodulatory receptor expression in the cortex.

The PI has a faculty mentoring committee, [redacted]. We meet annually to discuss career progress, grant ideas, and the like.

The [redacted] Institute and [redacted] Medical have core facilities for microscopy, histology, solution preparation, autoclaving, and statistics, and the Division of Laboratory Animal Resources performs a number of services for ordering, housing, and caring for laboratory animals. Our histological experiments to verify electrode positions have been performed in the [redacted] Medical histology core.

Equipment

Almost all of the major equipment required for these experiments has been obtained and is already operational in the lab. This includes:

Two *in vivo* recording rigs for extracellular and whole-cell recordings in awake, head-fixed animals. We have two 8' x 6' double-walled acoustic isolation chambers within the main lab space. Inside each is an air table for mechanical isolation (TMC), a surgical microscope (Zeiss), a stereotax (Kopf), a micromanipulator (Sutter MP-285), a fiber-optic lamp for illumination (Fisher), and a pre-amplifier (WPI). The pre-amp is connected to the data collection system outside the sound booth, consisting of a digitizer (Molecular Devices Digidata), a multichannel whole-cell recording amplifier (Molecular Devices MultiClamp), a PC with terabyte external hard drive for data storage, a current generator for electrical stimulation (Grass), and a digital signal processor for acoustic stimulus generation (TDT RZ6) connected to a speaker within the sound booth. We have several custom-built clamp systems for training and recording from awake, head-fixed mice, and a tabletop laser for optogenetic stimulation (CrystaLaser).

One 2-photon imaging system for imaging Ca^{2+} signals in awake, head-fixed animals, with a Movable Objective Microscope for imaging from auditory cortex (Sutter) and a Mai Tai Deepsee laser (Spectra-Physics). This imaging system is in an environmentally controlled annex down the hall from the main lab space. Our 2-photon microscope is dedicated for use to only our lab members, and contains a whole-cell recording setup for in vivo electrophysiology (as above) combined with 2-photon imaging.

One system for multielectrode recording from behaving animals (Blackrock). This system is designed for simultaneous extracellular recording with 16- or 32-channel chronically-implanted multielectrode arrays and op-tetrodes containing optical fibers. This system includes a PC for data analysis and storage, and a commutator for recording from animals within the behavioral testing chambers.

Three additional systems for stereotaxic in vivo surgeries and injections. Given the large number of surgeries, implantations, and injections we perform, we have three additional setups involving minimal recording but enabling users to stereotaxically inject or implant animals as needed, including acoustic stimulation (sound delivery) and extracellular multi-unit recording systems for verification of primary auditory cortex in head-fixed mice.

We have an electrode puller (Sutter P1000) in the main lab space. We have two refrigerators, a freezer, and two fume hoods for chemical and solution storage, and an analytical balance (Fisher) to aid in making internal solution for whole-cell recording. We have two isoflurane vaporizers (VetEquip).

We have access to the [redacted] histology core, containing a cryomicrotome for tissue sectioning, and microscopes for imaging fixed tissue on slides.

For these reasons, we are asking only for a limited amount of funding for equipment in this proposal. We collected pilot data on targeted whole-cell recordings under 2-photon control with amplifiers and manipulators taken from other rigs, and so we are requesting funds in year 2 to purchase additional manipulators to perform these key experiments. Additionally, while the scan mirrors in our 2-photon system have been sufficient to obtain preliminary data for this proposal, our imaging experiments- particularly axonal imaging- would benefit greatly by using resonant galvo scanners to increase the temporal resolution. Thus in year 3 we are requesting funds for these scanners to improve our data collection rates.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Robert Middle Name C Last Name*: Froemke Suffix:

Position/Title*: Assistant Professor
Organization Name*: [redacted]
Department: Otolaryngology
Division: [redacted]
[redacted]
Street2:
City*: [redacted]
[redacted]
[redacted]
Province:
Country*: USA: UNITED STATES
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: Ph.D. Degree Year: 2004

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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: Froemke, Robert Crooks

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

POSITION TITLE: Assistant Professor of Otolaryngology, Neuroscience & Physiology, Skirball Institute, NYU School of Medicine; Center for Neural Science, NYU

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
Tufts University, Medford MA	B.A.	11/1998	Computer Science
University of California, Berkeley CA	Ph.D.	05/2004	Molecular & Cell Biology
University of California, San Francisco CA	Postdoctoral	04/2010	Neuroscience, Otolaryngology

A. Personal Statement

We study neuromodulation, cortical plasticity, and auditory perception in rats and mice. I have a broad background in systems neuroscience, performing my undergraduate work at Tufts on machine learning and building modeling tools for complex systems analysis. For my PhD work with [redacted] at UC Berkeley, I examined spike-timing-dependent plasticity induced by natural spike trains in cortical networks. My postdoctoral research with [redacted] at UCSF focused on synaptic plasticity in vivo as related to auditory perception and behavior. As PI or co-Investigator on several university- and NIH-funded grants (including a K99/R00 and an R01), I have performed studies on auditory perception and synaptic plasticity in rodents for 12+ years. I have successfully administered the projects, trained several students (graduating three PhDs and one MD/PhD), postdocs, and technicians, collaborated with many other researchers, and produced several peer-reviewed publications from each project. This includes several studies examining the relation between excitatory-inhibitory balance, cholinergic/noradrenergic modulation, long-term synaptic plasticity, and auditory perception in rodents that the proposed studies directly extend. As a result of these experiences, I am aware of the importance of frequent communication with lab members and collaborators for constructing a realistic research plan and timeline, working towards our scientific goals. The current application builds logically on my prior work, and uses cutting-edge approaches to examine neuromodulation and synaptic plasticity in the context of controlled auditory behavior and psychophysical testing in mice.

1. Marlin BJ, Mitre M, D'amour JA, Chao MV, **Froemke RC**. Oxytocin enables maternal behaviour by balancing cortical inhibition. **Nature** 2015; 520:499-504. PMID: 25874674 PMC: 4409554
2. Martins ARO, **Froemke RC**. Coordinated forms of noradrenergic plasticity in the locus coeruleus and primary auditory cortex. **Nature Neuroscience** 2015; 18:1483-1492. PMID: 26301326 PMC: 4583810
3. D'amour JA, **Froemke RC**. Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. **Neuron** 2015; 86; 514-528. PMID: 25843405 PMC: 4409545
4. **Froemke RC**, Carcea I, Barker AJ, Yuan K, Seybold B, Martins ARO, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE. Long-term modification of cortical synapses improves sensory perception. **Nature Neuroscience** 2013; 16:79-88. PMC: 3711827

B. Positions and Honors

Positions and Employment

2010- Assistant Professor, New York University School of Medicine, Skirball Institute Program in Molecular Neurobiology; Neuroscience Institute; Departments of Otolaryngology, Neuroscience & Physiology; Kimmel Center for Stem Cell Biology; Center for Neural Science.

Other Experience and Professional Memberships

1996-1998 Senior research staff, Center for Connected Learning and Computer-Based Modeling
2000 Neural Systems & Behavior Course, Marine Biological Laboratory, Woods Hole, MA
2000- Member, American Association for the Advancement of Science
2000- Member, Society for Neuroscience
2004 Grass Fellow, Marine Biological Laboratory, Woods Hole, MA
2005- Member, Association for Research in Otolaryngology
2007 Okinawa Computational Neuroscience Course, Okinawa, Japan
2008 Methods in Computational Neuroscience, Marine Biological Laboratory, Woods Hole, MA
2009 Teaching Assistant, Biology of Memory Course, Cold Spring Harbor Laboratory, NY
2010 Kavli Institute for Theoretical Physics, UC Santa Barbara
2013 Co-Director, Biology & Disorders of Learning & Memory Course, Cold Spring Harbor Lab, NY
2013-2015 Co-Chair, Cosyne Workshop Committee (2 year appointment)

Honors

1998 Benjamin Brown Prize in Computer Science, Tufts University
2001 Howard Hughes Medical Institute Predoctoral Fellowship
2002 Outstanding Graduate Student Instructor, University of California, Berkeley
2004 First place, General Scientific Meeting presentation, Marine Biological Laboratory
2005 Jane Coffin Childs Postdoctoral Fellowship
2006 Sandler Translational Research Postdoctoral Fellowship
2008 K99/R00 Career Award, NIDCD
2011 Whitehead Fellowship
2012 Alfred P. Sloan Research Fellowship Award
2012 Pew Scholar Award
2012 Klingenstein Fellowship Award
2013 NYU Grand Challenge Award
2013 Hirschl/Weill-Caulier Career Award
2014 McKnight Scholar Award
2015 NYU “Next Gen Stars” Inaugural Speaker
2016 HHMI Faculty Scholars Award

C. Contribution to Science

1. We study the organization and plasticity of cortical synapses, and how cortical modulation and plasticity can improve auditory perception and behavior, in the context of behavioral training and perceptual learning. We have a particular emphasis on conducting well-controlled and parametric studies of auditory psychophysics, combined with mechanistic studies of neuromodulation and long-term plasticity of excitatory and inhibitory synapses. We have conducted a series of studies examining how manipulations of modulatory systems- the cholinergic attentional system of the nucleus basalis and the noradrenergic arousal system of the locus coeruleus - lead to synaptic plasticity and produce behavioral changes in adult rats. We assessed baseline auditory abilities to determine which stimuli were difficult to perceive, and leveraged the cholinergic system to boost up the strengths of synapses at these thresholds. We found that auditory perception was improved for at least hours afterwards, indicating that direct cortical modifications can be useful for enhancing sensory perception and behavior. We have contrasted these changes with the action of noradrenalin and stimulation (electrical or optogenetic) of the rat locus coeruleus, identifying how cholinergic and noradrenergic modulation differentially affect plasticity and perception. This is some of the first work identifying inhibitory synapses and circuits as central targets of neuromodulators, and our findings that acetylcholine disinhibits auditory cortex has been replicated by several other labs, in rats and in mice, and other sensory systems.

a. **Froemke RC**, Merzenich MM, Schreiner, CE. A synaptic memory trace for cortical receptive field plasticity. **Nature** 2007; 450:425-429. PMID: 18004384 PMC in process
b. **Froemke RC**, Carcea I, Barker AJ, Yuan K, Seybold B, Martins ARO, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE. Long-term modification of cortical synapses improves sensory perception. **Nature Neuroscience** 2013; 16:79-88. PMC: 3711827
c. **Froemke RC**. Plasticity of cortical excitatory-inhibitory balance. **Annual Review of Neuroscience** 2015; 38:195-219. PMID:25897875 PMC:4652600
d. Martins ARO, **Froemke RC**. Coordinated forms of noradrenergic plasticity in the locus coeruleus and primary auditory cortex. **Nature Neuroscience** 2015; 18:1483-1492. PMID:26301326 PMC: 4583810

2. Excitatory-inhibitory balance is an important property of mature neural circuits, ensuring that excitability is carefully controlled for information processing without seizure generation or propagation failure. How are inhibitory inputs calibrated during development and adjusted throughout life, to ensure that inhibition balances excitation? Inhibitory maturation is believed to determine critical periods for cortical development, and changes to excitatory synapses related to learning must be matched by coordinated changes in co-tuned inhibitory inputs. Our lab specializes in examining combined forms of excitatory and inhibitory long-term synaptic plasticity with whole-cell recordings and 2-photon imaging in vivo, monitoring the dynamics by which excitation and inhibition are adjusted after changes in patterns of electrical activity or sensory experience. We have shown how these processes occur during a critical period for frequency tuning in the rodent auditory cortex. We are also the first and perhaps only group to show how, after transplantation, inhibitory progenitor cells integrate into the existing network to open a new critical period in adult visual cortex, using paired recordings from host and transplanted cells to show that these new neurons make and receive synaptic connections in the local circuit.
   a. Dorrn A, Yuan K, Barker AJ, Schreiner CE, **Froemke RC**. Developmental sensory experience balances cortical excitation and inhibition. **Nature** 2010; 465:932-936. PMID:20559387 PMC2888507
   b. Southwell DG, **Froemke RC**, Alvarez-Buylla A, Stryker MP, Gandhi SP. Cortical plasticity induced by inhibitory neuron transplantation. **Science** 2010; 327:1145-1148. PMID:20185728 PMC:3164148
   c. Southwell DG, Paredes MF, Galvao RP, Jones DL, **Froemke RC**, Sebe JY, Alfaro-Cervello C, Garcia-Verdugo JM, Baraban SC, Alvarez-Buylla A. Intrinsically determined cell death of developing cortical interneurons. **Nature** 2012; 491:109-113. PMID:23041929 PMC:3726009
   d. Cohen S, Ma H, Kuchibhotla K, Watson BO, Buzsáki G, **Froemke RC**, Tsien RW. Excitation-transcription coupling in parvalbumin-positive interneurons employs a novel CaM Kinase-dependent pathway distinct from excitatory neurons. **Neuron** 2016; 90:292-307. PMID:27041500 PMC:4866871

3. Our studies of neuromodulation and behavior also examine circuit dynamics and the control of social cognition, with a particular emphasis on oxytocin. It has historically been difficult to determine how modifications of specific synapses relate to changes in behavior. We have examined how neurons in the rodent hypothalamus affect synaptic transmission in the cortex and elsewhere to produce behavioral changes in adult rats and mice. This is some of the first work using cortical plasticity to persistently enhance sensory perception and cognition. Furthermore, we provided the first direct evidence that oxytocin transiently reduces synaptic inhibition in the cortex, increasing the salience of incoming sensory inputs. We have used optogenetics and pharmacological approaches to examine how oxytocin can enable newly-maternal mice to recognize the significance of infant vocalizations and distress calls. As part of our work on oxytocin, in collaboration with Moses Chao's lab we generated the first specific antibodies to the mouse oxytocin receptor, which we have shared with many international laboratories (including the Grinevich and Stoop labs). We have also worked to understand the functional anatomy and circuit logic by which oxytocin neurons and other hypothalamic cell types project to target areas.
   a. Marlin BJ, Mitre M, D'amour JA, Chao MV, **Froemke RC**. Oxytocin enables maternal behaviour by balancing cortical inhibition. **Nature** 2015; 520:499-504. PMID: 25874674 PMC: 4409554
   b. Mitre M, Marlin BJ, Schiavo JK, Morina E, Norden S, Hackett TA, Aoki C, Chao MV, **Froemke RC**. A distributed network for social cognition enriched for oxytocin receptors. **Journal of Neuroscience** 2016; 36:2517-2535. PMID: 26911697 PMC: 4764667
   c. Eliava M, Melchior M, Knobloch-Bollmann S, Wahis J, da Silva Gouveia M, Tang Y, Ciobanu AC, del Rio RT, Roth LC, Althammer F, Chavant V, Goumon Y, Gruber T, Petit-Demoulière M, Busnelli M, Chini B, Tan L, Mitre M, **Froemke RC**, Chao MV, Giese G, Sprengel R, Kuner R, Poisbeau P, Seeburg PH, Stoop R, Charlet A, Grinevich V. A new population of parvocellular oxytocin neurons controlling

magnocellular neuron activity and inflammatory pain processing. **Neuron** 2016; 89:1291-1304. PMID: 26948889

d. Wong LC, Wang L, Yumita T, D'amour JA, Chen G, Chang B, Bernstein H, You X, Feng J, **Froemke RC**, Lin D. Effective modulation of male aggression through the lateral septum to medial hypothalamus projection. **Current Biology** 2016; 26:593-604. PMID: 26877081 PMC: 4783202

4. Neural activity can be complex. We have examined spike-timing-dependent plasticity (STDP), focusing on synaptic modifications induced by naturalistic patterns of pre- and postsynaptic spikes recorded in vivo. From hundreds of experiments we could predict the sign and magnitude of long-term synaptic plasticity induced by complex spike trains, and continue to work with computational neuroscientists to understand how STDP might enable neural networks to store and recall information. Some experiments examine how synaptic integration and dendritic properties affect NMDA receptor activation to control induction of synaptic plasticity. More recently, we are examining how multiple synapses are co-modified, and we were the first group to show that excitatory and inhibitory STDP can be coordinated and induced together. We found that spike pairing can normalize the strength of inhibition relative to the strength of co-activated excitation, providing a natural mechanism by which excitatory-inhibitory balance can be established and maintained.
   a. **Froemke RC**, Dan Y. Spike-timing-dependent synaptic modification induced by natural spike trains. **Nature** 2002; 416:433-438. PMID: 11919633
   b. **Froemke RC**, Poo MM, Dan Y. Spike-timing-dependent plasticity depends on dendritic location. **Nature** 2005; 434:221-225. PMID: 15759002
   c. Ponte Costa R, **Froemke RC**, Sjöström PJ, van Rossum MC. Unified pre- and postsynaptic long-term plasticity enables reliable and flexible learning. **eLife** 2015; 4. PMID: 26308579 PMC:4584257
   d. D'amour JA, **Froemke RC**. Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. **Neuron** 2015; 86:514-528. PMID: 25843405 PMC: 4409545

5. Studies of plasticity in the auditory cortex have the potential to inform and transform training procedures and use of neuroprosthetic devices such as cochlear implants. Our lab has pioneered a new rat model of multi-channel cochlear implant use, behaviorally and physiologically validated. This is being combined with µ-ECoG recordings for stable, long-term monitoring of cortical changes with use of the implants.
   a. Wang J, Trumpis M, Insanally M, **Froemke R**, Viventi J. A low-cost, multiplexed electrophysiology system for chronic µECoG in rodents. Conf Proc IEEE Eng Med Biol Soc 2014; 2014:5256-5259. PMID: 25571179 PMC: in process
   b. King J, Insanally M, Jin M, Martins ARO, D'amour JA, **Froemke RC**. Rodent auditory perception: critical band limitations and plasticity. **Neuroscience** 2015; 296:55-65. PMID: 27281743
   c. Insanally M, Trumpis M, Wang C, Chiang CH, Woods V, Bossi S, **Froemke RC**, Viventi J. A low-cost, multiplexed µECoG system for long-term, reliable high-density recordings in rodents. **Journal of Neural Engineering** 2016; 13:026030. PMID: 26975462, PMCID: in process.
   d. King J, Shehu I, Roland JT, Svirsky MA, **Froemke RC**. A physiological and behavioral system for hearing restoration with cochlear implants. **Journal of Neurophysiology** 2016; in press.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/robert.froemke.1/bibliography/44917017/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

HHMI Faculty Scholars Award 2016-2021
Cortical plasticity and control of social behavior
This career award supports our research on the mammalian oxytocin system, and cortical plasticity for learned maternal behavior. Role: PI

NYU CTSI Collaborative Translational Pilot Project Program 2016-2017
Optimizing cochlear implant use with neural recordings
The goal of this study is to understand the neural basis of cochlear implant use in trained, deafened rats. Role: Co-PI (with Mario Svirsky, NYU)

NYU Global Seed Grant for Collaborative Research 2016-2018
Neuromodulatory influences on economic preferences
The goal of this study is to examine the neurobiological mechanisms for impulsivity behaviors in trained rats. Role: Co-PI (with Jeff Erlich, NYU Shanghai)

Simons Foundation Explorer Grant 2016-2017
Synthetic infrared nanosensors for real-time monitoring of oxytocin release
The goal of this study is to screen carbon nanotube-based sensors for specific detection of oxytocin in real time. Role: Co-PI (with Markita Landry, UC Berkeley)

McKnight Scholar Award 2014-2017
Neural circuitry and plasticity for control of mammalian social behavior
The goal of this study is to determine the 'social receptive field' of oxytocin neurons in the hypothalamus, and relate activation of the oxytocin system and cortical plasticity to learned maternal behavior. Role: PI

Hirschl/Weill-Caulier Career Award 2014-2018
Neural basis of long-term memory formation
The goal of this study is to examine how neuromodulatory circuits of the locus coeruleus are modified by sensory experience and neural activity. Role: PI

R01 NIDCD DC012557 2012-2017
Synaptic basis for perceptual learning in primary auditory cortex
The goal of this study is to directly examine the relation between adult cortical synaptic plasticity and perceptual learning via the noradrenergic modulatory system. Role: PI

Completed Research Support

K99/R00 NIDCD DC009635 2008-2015
Synaptic basis for perceptual learning in primary auditory cortex
The goal of this study was to directly examine the relation between adult cortical synaptic plasticity and perceptual learning via the cholinergic modulatory system. Role: PI

Pew Scholarship 2012-2016
Neural basis of learned social behavior
The goal of this study was to determine how oxytocin affects responses to pup calls in the auditory cortex. Role: PI

Klingenstein Fellowship 2012-2015
Plasticity of excitatory-inhibitory balance in the auditory cortex
The goal of this study was to determine the network dynamics and mechanisms that calibrate and balance excitation and inhibition in the developing auditory cortex. Role: PI

Alfred P. Sloan Research Fellowship 2012-2014
Synaptic plasticity in the cerebral cortex
The goal of this study was to examine the functional consequences of long-term synaptic plasticity in cortical networks for behavioral modification. Role: PI

NYU Provost's Grand Challenge Grant 2013-2015
Smart neuroprosthetics: brain-machine interfaces for the 21st century
The goal of this study was to generate brain-machine interface devices for improving sensory, motor, and cognitive behavioral performance in rodents and primates. Role: Co-PI (with Michael Long, Bijan Pesaran, Dan Sanes, and Jonathan Viventi, NYU)

NYU Provost's Office Mega-Grants Initiative 2013-2014
Creating a neuroengineering core facility at NYU
The goal of this study was to seed construction of a core facility at NYU for fabrication of neuroprosthetic devices. Role: Co-PI (with Michael Long, Dan Sanes, John Simspon, and Jonathan Viventi, NYU)

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: [redacted]

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Robert	C	Froemke		PD/PI	[redacted]	5	0	0	[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	15	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]				[redacted]		[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	[redacted]
Total Equipment	[redacted]

Additional Equipment: File Name:

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

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

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

Tracking Number: GRANT12208477 Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	0.00

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research (MTDC)	[redacted]	[redacted]	[redacted]
		[redacted]	[redacted]

Cognizant Federal Agency DHHS, [redacted]
(Agency Name, POC Name, and POC Phone Number)

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

J. Fee	Funds Requested ($)*
	[redacted]

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

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: [redacted]

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Robert	C	Froemke		PD/PI	[redacted]	5	0	0	[redacted]	[redacted]	[redacted]
[redacted]													

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]							
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]							

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	[redacted]
Total Equipment	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research (MTDC)	[redacted]	[redacted]	[redacted]
		[redacted]	[redacted]

Cognizant Federal Agency DHHS, [redacted]
(Agency Name, POC Name, and POC Phone Number)

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

J. Fee	Funds Requested ($)*
	[redacted]

K. Budget Justification* File Name: budget_justification_final.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*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: [redacted]

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Robert	C	Froemke		PD/PI	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]													
[redacted]													

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]				[redacted]	[redacted]	[redacted]
					[redacted]		[redacted]

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	[redacted]
[redacted]	[redacted]

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

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

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

Tracking Number: GRANT12208477 Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research (MTDC)	[redacted]	[redacted]	[redacted]
		[redacted]	[redacted]

Cognizant Federal Agency DHHS, [redacted]

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

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

J. Fee	Funds Requested ($)*
	[redacted]

K. Budget Justification* File Name: budget_justification_final.pdf

(Only attach one file.)

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

Tracking Number: GRANT12208477 Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: [redacted]

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Robert	C	Froemke		PD/PI	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]													
[redacted]													

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]				[redacted]		[redacted]
					[redacted]		[redacted]

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	[redacted]
[redacted]	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

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

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

Tracking Number: GRANT12208477 Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research (MTDC)	[redacted]	[redacted]	[redacted]
		[redacted]	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl W. Mayes, 212-264-2069

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

J. Fee	Funds Requested ($)*
	[redacted]

K. Budget Justification* File Name: budget_justification_final.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*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: [redacted]

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Robert	C	Froemke		PD/PI	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]													
[redacted]													

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12	0	0	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]				[redacted]		[redacted]
					[redacted]		[redacted]

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

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

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

ORGANIZATIONAL DUNS*: 121911077

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: New York University School Of Medicine

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	[redacted]
[redacted]	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: [redacted]

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research (MTDC)	[redacted]	[redacted]	[redacted]
		[redacted]	[redacted]

Cognizant Federal Agency DHHS, Darryl W. Mayes 212-264-2069
(Agency Name, POC Name, and POC Phone Number)

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

J. Fee	Funds Requested ($)*
	[redacted]

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

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

Budget Justification
The high cost of living [redacted] necessitates salaries that are higher than elsewhere in the US. This project requires our participation, and the full-time effort of lab members in the [redacted] lab. These salaries alone, together with fringe benefits, would almost be the maximum budget for a modular grant. We are requesting minimal equipment to perform the Aims of this study, but costs for essential supplies and mice increase the total budget to [redacted] As this is our renewal application of our sole R01, we have no other support to study how cholinergic and noradrenergic neuromodulation enable behaviorally-relevant plasticity in the auditory system.

Personnel
Dr. Robert C. Froemke (PI, 5 months effort) will be responsible for administrating this project, determining the directions, establishing priorities for experiments and coordinating research efforts with lab members. He will perform some in vivo recording and behavioral experiments, especially at the outset, and train personnel.

[redacted] a postdoctoral associate (3 months effort in year 1, zero salary) will be responsible for training the graduate students and technicians on surgical procedures, recording and two-photon imaging in awake head-fixed mice. [redacted] has extensive experience in 2-photon imaging of cortical activity in awake head-fixed mice, having earned his Ph.D. under the supervision of [redacted] His thesis work showed how visual cortical processing is disrupted by pathologies such as epilepsy and Alzheimer's disease, and thus he is highly trained in performing surgeries; building and using a 2-photon microscope; generating, calibrating, and controlling presentation of sensory stimuli; and data analysis with Matlab and other programs. In graduate school, [redacted] was supported by an NIH NRSA Predoctoral Fellowship, a James Mills Pierce Scholarship from Harvard Medical School, and won the Best Paper in Neuroimaging Award at the International Alzheimer's Disease conference. He had a first-author paper in Science and several other publications as a graduate student, and has built our 2-photon system. As shown by our preliminary data and our co-authored publication in Neuron (Cohen et al., Neuron 2016), we are already acquiring stable imaging data from awake head-fixed mice and performing 2-photon guided whole-cell recordings. He joined the Froemke lab in 2012; his salary is [redacted], but we are requesting no salary support as [redacted] has a K99/R00 award.

TBD, a postdoctoral associate (12 months effort), will be trained [redacted] on imaging and recording procedures in head-fixed mice.

[redacted] a [redacted] PhD graduate student (9 months effort), will perform most of the behavioral studies and in vivo recordings, focusing on cortex. [redacted] got her BS in Industrial Biotechnology from the [redacted] where she founded an undergraduate program in neuroscience (Neuro-RUM) across multiple UPR campuses. [redacted] will begin as a graduate student Fall 2016 and been working in the Froemke lab as a research assistant during the summers for the past 2 years. She is already working in our lab collecting pilot data for this proposal. Her salary is [redacted]

[redacted] a technician (12 months effort), will perform some behavioral experiments (including implantation of head-posts and training mice to head fix), help with anatomical and immunohistological studies, assist with recovery surgeries, and monitor implanted animals. [redacted] has been trained in the Froemke lab in these techniques since Spring 2015. She recently graduated from Teachers College [redacted] with a MS in Neuroscience and Education. Her salary is [redacted]

Equipment
To perform stereotaxic injections and implantations, as well as 2-photon targeted whole-cell recordings in awake animals, we are requesting funds to purchase additional manipulators in year 2 [redacted] This equipment is required for this study, given the importance of whole-cell recordings and viral injections for GCaMP6s or opsin expression. We also request funds to purchase resonant galvo scanners in year 3 [redacted], which will greatly increase the temporal resolution of imaging, especially important for the 2-photon imaging of axon fibers.

Supplies
We are requesting funds to purchase and house animals, and order viral constructs, anesthetic agents and other pharmacological reagents, and surgical supplies and electrodes.

Travel

We are requesting funds to support the PI's travel to two meetings each year (the Society for Neuroscience annual meeting and either Cosyne or the Association for Research in Otolaryngology midwinter meeting), as well as support the attendance of the postdocs and graduate students at one meeting each year.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
[redacted]	[redacted]
Total Number Other Personnel	16
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
1. Domestic	[redacted]
[redacted]	[redacted]
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	[redacted]
[redacted]	[redacted]
8. Other 1	0.00
9. Other 2	0.00
10. Other 3	0.00
Section G, Direct Costs (A thru F)	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

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

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

3. *Program Income Section

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

○ Yes ● No

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

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

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ● No

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

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12208477 Funding Opportunity Number: PA-16-160 . Received Date: 2016-07-05T15:19:02.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	aims_final.pdf
3. Research Strategy*	research_strategy_final.pdf
4. Progress Report Publication List	progress_report_publication_list.pdf
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	animals_final.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	support_letters_final2.pdf
14. Resource Sharing Plan(s)	resource_sharing_final.pdf
15. Authentication of Key Biological and/or Chemical Resources	resource_authentication_final.pdf
Appendix	
16. Appendix	

Tracking Number: GRANT12208477
Funding Opportunity Number: PA-16-160. Received Date: 2016-07-05T15:19:02.000-04:00

I. Specific Aims

Synaptic basis of perceptual learning in primary auditory cortex

The goal of this project is to determine how cholinergic and noradrenergic modulatory systems collectively promote synaptic plasticity in primary auditory cortex (AI), to improve auditory perceptual abilities in behaving mice. Modulation and plasticity are important features of AI, especially for forming representations of sensory signals such as speech, music, or other behaviorally-relevant sounds[1-10]. Changes in neural circuits and behavior can be incredibly long-lasting after auditory conditioning[11-14], but the mechanisms by which AI networks are persistently modified and affect auditory perception are unclear. In particular, it remains challenging to connect perceptual learning to plasticity in AI or elsewhere in the brain. Impaired cortical processing and plasticity are believed to occur in disorders such as hearing loss, language impairments, and tinnitus[15-17]; conversely, enabling plasticity by training programs and devices such as hearing aids and cochlear implants may improve outcomes in pathological conditions and hearing loss[18-22]. Successful completion of this project will result in a significantly improved mechanistic model of auditory learning and AI plasticity, critical for studies of language learning, speech processing, deafness, and the design of medical devices and training procedures for hearing restoration.

Adult cortical plasticity requires sensory experience and neuromodulation, which relays global behavioral context to local cortical circuits. Many modulatory systems, including the noradrenergic system of the locus coeruleus[23-30] and the cholinergic system of the basal forebrain[1,2,31-40], are recruited by surprising or arousing stimuli, and promote plasticity in target circuits including rodent AI. What are the functional differences between acetylcholine and norepinephrine for perceptual learning and auditory behavior? Specifically, here we will ask how training affects AI circuits during behavior (Aim 1); if cholinergic and noradrenergic systems are recruited at distinct times, to differentially modulate AI during auditory training for enhanced perception (Aim 2); and finally, when and how neuromodulatory neurons become responsive to task-relevant sounds and other cues during training (Aim 3).

Previously we studied cholinergic or noradrenergic modulation separately in behaving rats, measuring physiological changes under anesthesia[10,28,35,36]. Here we will relate behavior to neural activity with in vivo whole-cell recording and 2-photon imaging from AI and modulatory neurons in behaving head-fixed mice. These studies take advantage of our past work on AI modulation and plasticity[28,35,36,41-45], using a novel approach we developed to document synaptic plasticity in multiple cells over days, in behaving mice performing an auditory task. This allows us to measure and manipulate modulatory systems on a trial-by-trial basis while monitoring responses over the entire time-course of learning in individual mice. We hypothesize that initially, norepinephrine reduces tonic inhibition to increase excitability to all stimuli; this may increase detection and behavioral responses but at the cost of false alarms. As mice learn the task over days, acetylcholine then selectively enhances a sub-population of excitatory and inhibitory AI neurons for reliable task performance. Our goal is a unified model of cholinergic and noradrenergic modulation for AI excitatory and inhibitory plasticity important for behavior.

Aim 1. To determine how AI excitatory/inhibitory cells and synapses are modified by auditory training

We will train adult mice on a go/no-go auditory task, to examine perceptual learning by measuring detection thresholds and sound recognition abilities. We will use in vivo 2-photon imaging (Subaim 1a) and whole-cell recordings (Subaim 1b) from AI neurons in behaving mice to determine how excitatory/inhibitory inputs and outputs are modified by learning, and assess if these changes are required for task performance.

Aim 2. To ask how cholinergic and noradrenergic modulation affect auditory learning and AI plasticity

We will record from AI during behavior, using optogenetics and pharmacology to ask if and when the cholinergic vs noradrenergic systems are necessary for initial learning and maintained peak performance (Subaim 2a). We will ask if pairing task-relevant sounds with cholinergic and/or noradrenergic modulation can accelerate learning or enhance peak performance (Subaim 2b). We will determine the synaptic mechanisms by which cholinergic and noradrenergic modulation enable AI excitatory/inhibitory plasticity during learning.

Aim 3. To test the hypothesis that noradrenergic and cholinergic neurons respond to task variables

How are the noradrenergic and cholinergic systems recruited when mice are engaged in the task? We will ask if there is a sequence of AI modulation over task learning; specifically if the noradrenergic system is recruited transiently during the first day, while the cholinergic system comes on-line over subsequent days and is needed for steady-state performance. We will record from locus coeruleus and nucleus basalis, and perform 2-photon imaging of modulatory axons within AI in trained animals and over the duration of perceptual learning. We also recently found that tone-evoked responses with short latency are induced in locus coeruleus neurons after pairing tones with locus coeruleus stimulation[28]. Here we will ask if plasticity in nucleus basalis and/or locus coeruleus is important for auditory learning and task performance.

II. Research Strategy

A. Significance

The auditory cortex is modulated and modified after significant experiences, learning meanings of words or other sounds, sensory deprivation, or use of devices such as cochlear implants[1-22]. Synaptic plasticity is thought to be a fundamental mechanism for long-term changes in network function. However, relations between modulation, plasticity, network reorganization, and changes in perception and behavior are complex and remain unclear.

This proposal describes a series of *in vivo* imaging and recording experiments in AI of behaving mice to reveal: 1) when and how auditory input interacts with the neuromodulators acetylcholine and norepinephrine (the main modulators of sensory attention) to modify AI responses, and 2) under what conditions these changes improve auditory perception. The main hypothesis is that the cholinergic and noradrenergic systems have different temporal dynamics and distinct effects on AI responses and perception (**Fig. 1a**). Specifically, we hypothesize that on the first training day, norepinephrine boosts AI and behavioral responses to all stimuli. This is important for animals to begin responding, to learn task variables and stimulus-response contingencies. As mice learn the task, cholinergic modulation then refines AI representations for reliable performance (in high-performers). We aim to document this process over the training duration with imaging and recordings in behaving mice (**Fig. 1b**).

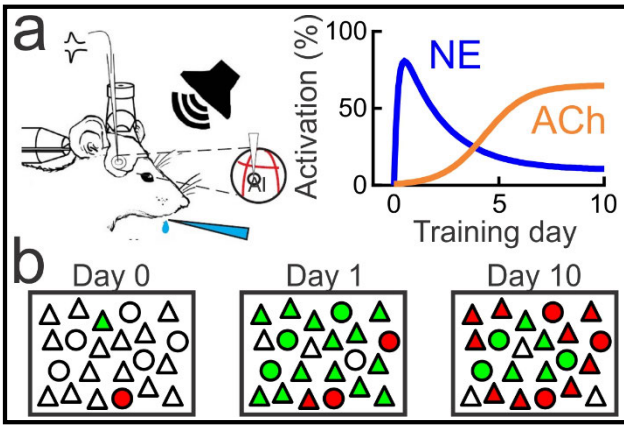

Figure 1. General hypotheses of this proposal. **a**, Left, experiment schematic- in vivo 2-photon imaging and whole-cell recording in mice performing an auditory detection and recognition task for water reward. Right, we predict that norepinephrine (NE) release is initially high immediately at start of training, but decays rapidly over days as acetylcholine (ACh) modulation becomes important for task performance. **b**, Network hypothesis: AI cells (triangle, excitatory; circle, inhibitory) are not initially positively (green) or negatively (red) modulated by task before learning begins. At Day 1, NE leads to large-scale activation of AI during task. By Day 10, ACh activates a sub-ensemble, but silences many other cells.

The overall goal of our research is to understand the circuit mechanisms of auditory perceptual learning. Long-term plasticity requires two factors: sensory experience and activation of neuromodulatory systems, which relay global behavioral context to local cortical circuits[1,2,10,11,46-51]. Sensory stimulation alone does not usually lead to positive long-term changes in adults without context-defining modulatory signaling. Conversely, modulation alone can have negligible effects or be potentially pathological without sensory input to guide plasticity[3,10,52-54].

Our lab has studied the complex interactions between these variables for years in the rodent auditory system, with behavioral training, electrophysiological recordings in vivo and in vitro, two-photon imaging, computational modeling, and anatomical/biochemical studies of modulatory systems[28,35,36,41-45,55-62]. We have examined how sensory input (e.g., tones, frequency modulated (FM) sweeps, vocalizations) interact with brain state (e.g., encoded by norepinephrine or acetylcholine), to induce long-term synaptic plasticity and affect perception. We previously examined cholinergic or noradrenergic systems separately, studying how these modulators improve (or degrade) auditory perception in behaving rats, while making whole-cell recordings in anesthetized animals to examine how modulation paired with sounds changes AI representations of paired or unpaired input[28,35,36].

In this proposal, we take the next logical steps, studying modulation, plasticity, and learning in the same animals. Specifically, in **Aim 1** we will perform 2-photon imaging and whole-cell recording from AI neurons in behaving mice to measure changes to synaptic input and spiking output. In **Aim 2** we ask if noradrenergic or cholinergic modulation of AI excitatory/inhibitory plasticity are required to learn and improve perceptual abilities. In **Aim 3**, we image and record from cholinergic and noradrenergic modulatory neurons during auditory training. We will relate spatio-temporal patterns of modulatory activity to the time course of auditory learning, and examine changes to neuromodulatory systems directly. Previous results[23,24,28,38,63-65] suggest modulatory cells can begin responding to sounds after conditioning, possibly important for prolonging AI plasticity and perceptual changes.

Auditory processing is context specific, reflecting the importance of perceptual salience and behavioral relevance for sensory perception[66-69]. Among other purposes, this allows humans and animals to focus on vocalizations or other behaviorally-important sounds to learn their meanings, recognize such sounds in complex and noisy acoustic environments, and increase arousal level during surprising or hazardous experiences. Studying auditory plasticity in AI is particularly important because contextual modulation of auditory processing is stronger in AI as compared to subcortical stations[36,70-73], and because it has been linked to the activity of neuromodulatory nuclei

that project to AI[28,29,30-36]. Electrical or optogenetic stimulation of these subcortical regions enables plasticity of AI tuning curves and auditory responses, influences perception, and modifies behavior, making neuromodulation a valuable tool for therapeutic purposes and for identifying mechanisms of plasticity in vivo[1,2,5,10,14,20,30-36,42,43,47].

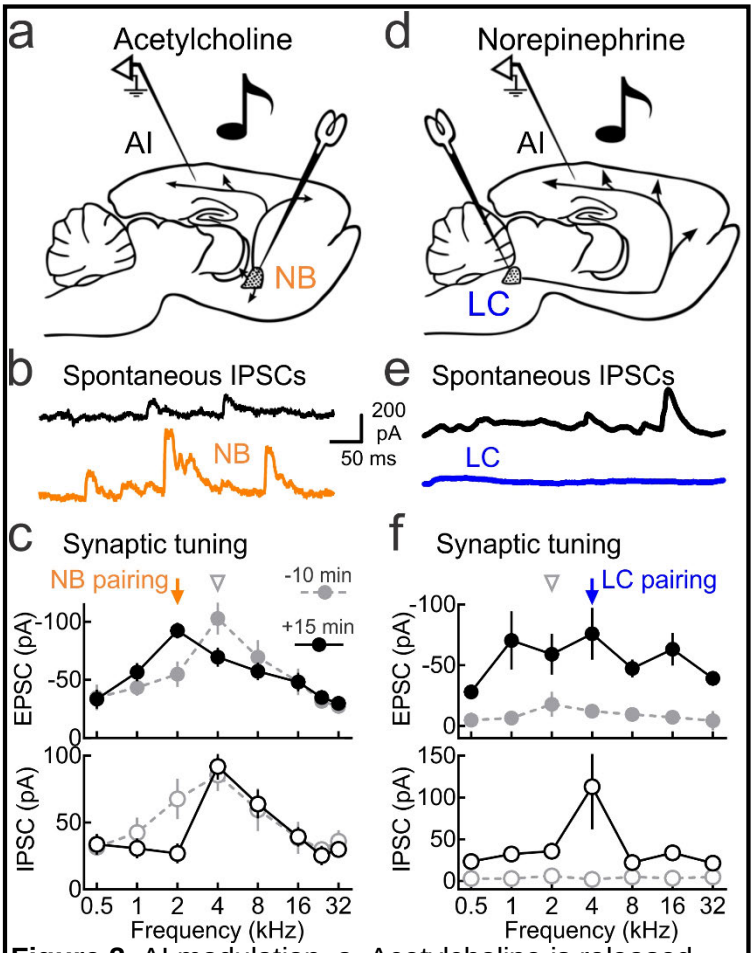

Figure 2. AI modulation. **a**, Acetylcholine is released into AI from nucleus basalis (NB). **b**, In vivo voltage-clamp recording in rodent AI; NB stimulation *increased* spontaneous IPSCs. **c**, NB stimulation paired with 2 kHz tone repetitively *reduced tone-evoked IPSCs* and enhanced paired tone EPSCs. **d**, Norepinephrine is released by locus coeruleus (LC). **e**, In contrast to NB, LC stimulation *reduced* spontaneous IPSCs. **f**, LC pairing enhanced tone-evoked EPSCs and IPSCs together. Note magnitude of increase (refs. 28,35).

Many neuromodulators affect cortical responses in some way, such as social salience cues provided by oxytocin[42,43,74] or reward prediction errors signaled by dopamine[75-78]. However, the two main modulators for attentional control of sensory processing are acetylcholine (released specifically into cortex from the nucleus basalis; **Fig. 2a-c**) and norepinephrine (from locus coeruleus and other brainstem centers; **Fig. 2d-f**). Nucleus basalis contains a distribution of cholinergic and non-cholinergic cells, important for selective attention to behaviorally-relevant stimuli[31-33,37-39,79]. Nucleus basalis projects mainly to cortex, with inputs from limbic areas and prefrontal cortex[31,39]. In contrast, locus coeruleus is a small brainstem area (~1-2k and 20-50k cells in rodents and humans, respectively) with a primary role in general arousal and selective attention to some degree. Locus coeruleus inputs include brainstem, limbic system and prefrontal cortex, and outputs are widely targeted throughout the brain, including AI and thalamus[25,26]. Both systems are implicated in many physiological and pathological aspects of brain function, including learning and memory, arousal, and addiction[26,29,32-36,47,64,,65,80]. Both systems degenerate early in Alzheimer's disease, possibly leading to the 'cognitive fog' in this condition[81,82]. Despite some similarities between cholinergic and noradrenergic systems, there are anatomical and functional distinctions perhaps important for context-dependent modulation and auditory learning. Locus coeruleus is hypothesized to convey 'top-down' uncertainty between actual and expected input, or 'bottom-up' alerting signals to surprising events. Cholinergic signaling might also convey this type of mismatch, but may be more important for 'expected uncertainty' (i.e., steady-state attention to learned environmental/behavioral contingencies). In contrast, norepinephrine signals 'unexpected uncertainty'[23-26,64,83,84] (i.e., at start of training or dramatic changes to environments or behavioral outcomes).

How do cholinergic and noradrenergic modulation affect AI synaptic responses? Many studies in vivo and in vitro show that cholinergic or noradrenergic agonists affect neuronal excitability, synaptic transmission, and enable plasticity; in contrast, receptor blockers prevent plasticity[28,36,47-51,85-94]. Over the last decade we have systematically studied cholinergic or noradrenergic systems in vivo (**Fig. 2**), examining how modulation affects spontaneous excitatory and inhibitory postsynaptic currents (E/IPSCs, **Fig. 2b,e**), tone-evoked responses and AI receptive fields at synaptic (**Fig. 2c,f**) and spiking levels (**Fig. 3**), and examined behavioral consequences of neuromodulatory pairing (electrically or optogenetically) and plasticity of AI frequency or intensity tuning (**Fig. 4**).

Both modulators reduce inhibition, but in different ways. Nucleus basalis stimulation in adult rats increased spontaneous IPSCs measured with in vivo whole-cell recordings (**Fig. 2b**, orange); while tone-evoked IPSCs were reduced (**Fig. 2c**), replicating past work[1]. This depends on muscarinic receptors as it was blocked by atropine, and induced NMDA receptor-dependent long-term potentiation (LTP) of evoked EPSCs (**Fig. 2c**). Importantly, excitation and inhibition are co-tuned and correlated (**Fig. 2c**, gray), or 'balanced' in adult AI[10,41,44,95-98]. Cholinergic disinhibition transiently disrupts excitatory-inhibitory balance, promoting tuning curve plasticity before inhibition re-balanced excitation after hours[35,36]. Synaptic changes induced by 5 min nucleus basalis

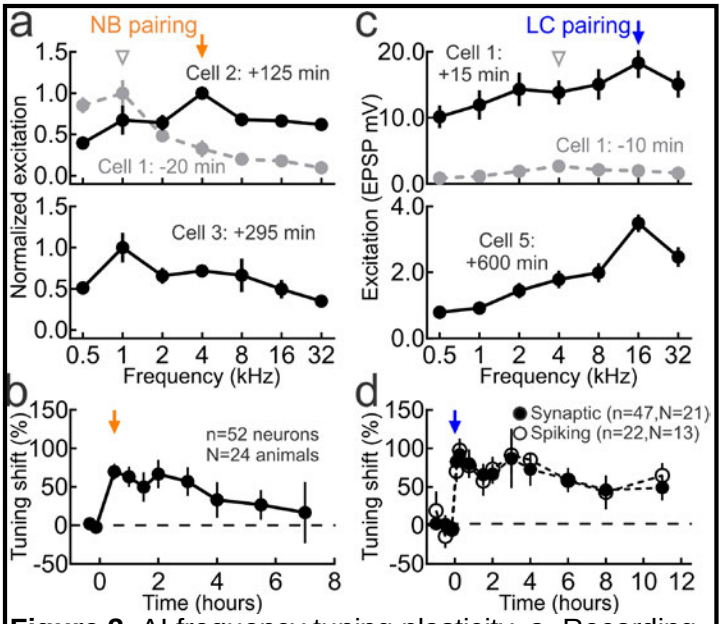

Figure 3. AI frequency tuning plasticity. **a**, Recording in 1 kHz region of AI (arrowhead); NB stim paired with 4 kHz (arrow). Tuning shifts lasted 1-3 hrs, but retuned to 1 kHz after ~6 hrs; tuning shift was x-axis peak translation. **b**, Summary of NB pairing shifts. **c**, LC pairing increased responses to all tones by ~10X, producing y-axis increase in response gain. After ~1-3 hrs responses recovered, but peak shift (16 kHz) endured. **d**, LC pairing effect duration (ref. 28).

pairing shifted tuning curves towards paired tones (**Fig. 3a**). However, >6 hrs without additional nucleus basalis stimulation, responses shifted back to the original tuning in register with AI tonotopy (**Fig. 3b**). In contrast, locus coeruleus pairing lowered spontaneous ISPCs (**Fig. 2e**). Reduced spontaneous inhibition greatly increased excitability for all frequencies[28], leading to large 200-1000% increases of tone-evoked excitation and inhibition together (**Fig. 2f,3c**). This nonspecific increase recovered, and tuning curves remained shifted to paired frequencies for 12+ hours, essentially persisting as long as measured (**Fig. 3d**). We measured perceptual effects of AI plasticity by performing behavioral studies[28,36], conditioning rats on a 'go/no-go' tone detection-recognition task, rewarding them for responding to 4 kHz targets (**Fig. 4a**). Nucleus basalis pairing selectively but transiently enhanced behavior (**Fig. 4b,c**). Locus coeruleus pairing initially impaired or did not affect performance, but after a few hours, detection and recognition were improved for days (**Fig. 4d,e**), similar to AI changes. Moreover, just one episode of locus coeruleus pairing accelerated learning rate when target frequency was switched[28].

Thus the major effects of cholinergic modulation are: reduced stimulus-evoked inhibition, producing hours-long shifts in tuning curve peaks while conserving total excitability (i.e., x-axis translations in AI tuning). In comparison, noradrenergic modulation leads to: reduced spontaneous inhibition but increased stimulus-evoked EPSCs/IPSCs, first leading to a large response gain to all stimuli (i.e., y-axis translation and broadening of AI tuning), before excitability recovers and long-lasting changes emerge. Pairing-induced changes in AI tuning are directly reflected in auditory behaviors, in terms of enhanced detection, improved (or worse) target recognition, and duration of these behavioral changes. These results suggest that cholinergic and noradrenergic modulation play a crucial role underlying auditory plasticity and learning. We hypothesize that even when these forms of modulatory pairing are not experimentally imposed (as in our past studies[28,35,36]), they are naturally engaged by perceptual training, which we will confirm by recording and imaging in behaving mice in **Aims 1** and **2**.

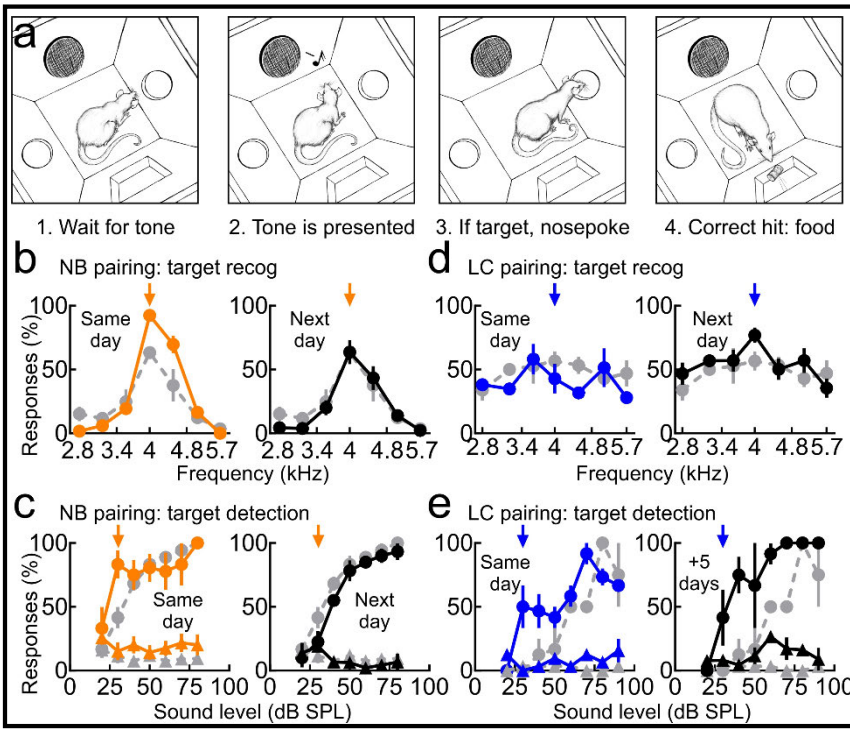

Figure 4. NB and LC pairing improve auditory behavior. **a**, Food-deprived rats were operantly conditioned to respond for food reward to target 4 kHz tones (any intensity), withholding responses to foils. We can systematically vary sound level to probe psychophysical detection thresholds (background noise level ~30 dB SPL), or adjust frequency range of foils to assess recognition abilities. **b**, NB pairing improved target recognition for hours (gray, baseline; orange, 1-2 hrs after pairing), but performance returned on the next day. **c**, NB pairing selectively improved detection of quiet tones at the paired intensity, but changes lasted only a few hrs. **d**, LC pairing initially had little effect on the same session, but led to selective and enduring changes thereafter. **e**, LC pairing greatly enhanced detection for 5-30 days (refs. 28,36).

We examined circuit mechanisms of persistent changes in AI responses and behavior after locus coeruleus pairing[28]. We recorded from locus coeruleus cells (**Fig. 5a**) and found these neurons were also plastic; short-latency tone responses (~50 msec from onset) emerged after pairing tones with locus coeruleus stimulation or foot shock. In vivo whole-cell recordings showed subthreshold responses developing in initially-unresponsive cells; plasticity was prevented with APV (NMDA receptor antagonist) infused into locus coeruleus (**Fig. 5b**). Preventing locus coeruleus plasticity reduced duration of AI tuning curve shifts (**Fig. 5c**) and perceptual improvement after pairing (**Fig. 5d**). This replicated and extended past studies showing locus coeruleus responses to conditioned stimuli after training[23,24]. Now in this proposal, we will ask in **Aim 3** if this 'plasticity of neuromodulation' naturally occurs in nucleus basalis and locus coeruleus during training, and how this control of modulation impacts AI plasticity and auditory perceptual learning in mice.

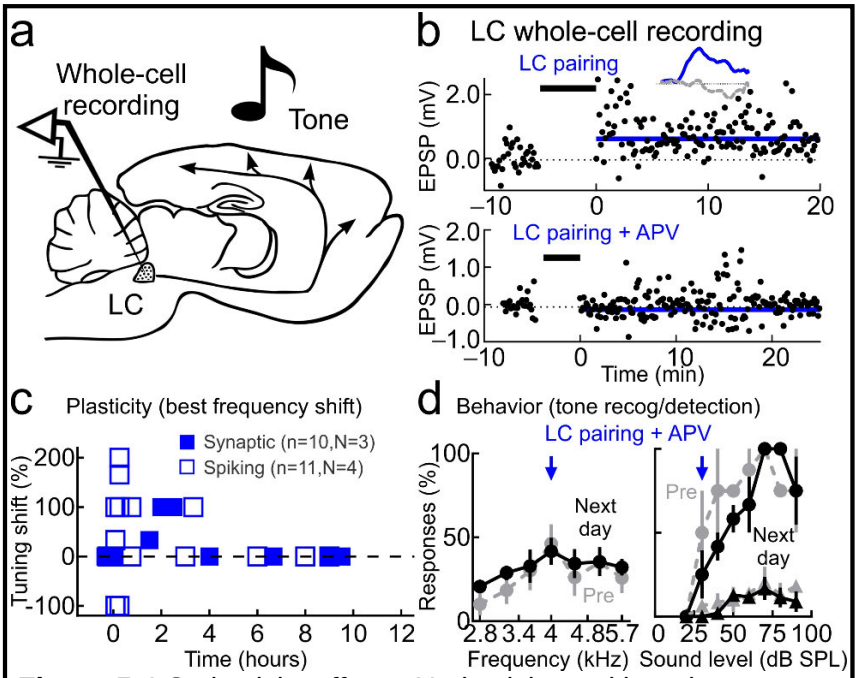

Figure 5. LC plasticity affects AI plasticity and learning. **a**, Recording schematic. **b**, In vivo whole-cell recordings from adult rat LC. Shown are membrane potential responses to paired tone. Top, tone-evoked responses increased from ~0.1 mV (noise level) to ~1.0 mV. Inset, EPSPs. Bottom, APV (1 mM in LC) prevents LC plasticity. **c**, Synaptic and spiking tuning shift duration is *reduced* to 1-3 hrs when LC plasticity is blocked w/ APV. **d**, Long-lasting behavioral changes are also prevented by APV infused into LC (c.f. **Fig. 4c,e**) (ref. 28).

Neuromodulation is widespread in the auditory system, important for attention, arousal, plasticity, and learning. There is a large literature on modulation of AI processing and plasticity[1-14], but direct connections between synaptic plasticity and perceptual learning are lacking. Our studies provide foundational data towards a general theory of cortical modulation and plasticity, examining cortical-subcortical interactions, and how training drives multiple modulatory systems to synergistically enhance sensory processing for improved perception. We expect these methods and results will apply to other neural systems, and contribute to studies of language learning and the role of attention vs. arousal in auditory processing. These studies will also inform training programs and rehabilitation strategies[15-22] for disorders such as hearing loss or tinnitus, and use of hearing aids or cochlear implants.

B. Innovation

This project is innovative in four main ways: 1) Connecting synaptic plasticity to behavioral changes is a central challenge in neuroscience. While training and experience affect neural circuits, we have shown how AI synaptic modifications causally improve behavior for hours, days, or weeks[28,35,36,42]. We are one of the few groups studying long-term excitatory/inhibitory plasticity in vivo with whole-cell recording (**Fig. 2**) and optogenetics. Here we extend this approach for head-fixed mice (**Fig. 6**), to leverage genetic advantages (particularly for imaging).

2) Perceptual learning can take days, but the duration of whole-cell recordings is much shorter. We are one of the only labs combining whole-cell recordings with 2-photon in vivo imaging to examine long-term synaptic plasticity in behaving mice, relating AI synaptic and spiking changes to task performance in behaving animals (**Figs. 2-5**). We refined methods for *in vivo* recordings from multiple cells over hours and days[28,35,36,41,42,61], and use 2-photon imaging[45,61] to monitor the same cells for weeks during behavior (**Fig. 7**) or image neuromodulatory axons within AI. This enables us to record and monitor behavioral changes as animals learn and task performance improves, and relate single-trial neural responses to behavioral performance in the same animals.

3) Our task is parametric, enabling us to perform careful psychophysical studies. We can control consequences of nucleus basalis and locus coeruleus pairing, by over- or under-representing particular paired or unpaired sounds[28,36]. This allows us to ask more directly how the neurometric functions relate to psychophysical abilities.

4) We are one of the only labs to record directly from modulatory neurons, including in vivo whole-cell recordings in locus coeruleus[28]. These experiments reveal that these neuromodulatory neurons rapidly develop sensory responses after pairing even at the subthreshold synaptic level, and govern auditory plasticity and perception.

C. Approach

Responses of central auditory neurons are changed by experience and training, but mechanisms and functional relations of synaptic plasticity to auditory perception are unclear. Here we will clarify how AI plasticity relates to enhanced auditory perception during training by in vivo imaging and recording in head-fixed behaving mice. Mice offer many advantages including optogenetics and ease of intracellular recordings in awake animals. The overall goal is to gain insight into functional differences between the cholinergic and noradrenergic modulatory systems, and how behavioral context interacts with experience to change auditory circuits for improved perception. The central hypothesis is that perceptual training refines AI representations through plasticity of local inhibition (**Aim 1**), driven initially by noradrenergic modulation before cholinergic tone increases to support task performance (**Aim 2**). Task variables also come to be represented by neuromodulatory neurons in order to appropriately regulate cortical modulation during initial learning, and then for continued reliable behavioral responses (**Aim 3**).

Aim 1. To determine how AI excitatory/inhibitory cells and synapses are modified by auditory training

We will train adult mice on a go/no-go auditory task, to examine detection thresholds and sound recognition abilities. We will use in vivo 2-photon imaging (Subaim 1a) and whole-cell recordings (Subaim 1b) from AI neurons in behaving mice to determine how excitatory/inhibitory inputs and outputs are modified by learning.

Subaim 1a. To determine the dynamics of AI excitatory and inhibitory cell responses during auditory training.

Our first goal is to adapt our go/no-go auditory detection/recognition task for head-fixed mice. We will use 2-photon imaging to monitor excitatory cells and inhibitory cell subclasses over the course of learning.

Methods: We will train male and female adult C57Bl6 mice (~20-30 g) on a go/no-go auditory detection-recognition task (**Fig. 6a**) where correct responses are licks after a target sound and withholding licking to other non-target/foil sounds. For some mice, tones-in-noise will be used, in other animals we will use FM sweeps. Stimuli will be 100-500 msec duration, 2-7 s inter-trial intervals. This design gives us a high level of control over task difficulty, varying signal-to-noise level or spectral similarity of targets vs foils. Mice will be water-restricted and trained in daily sessions of ~2 hrs each. Performance will be measured by hit, miss, correct withhold, and false alarm rates, and number of trials/time to criterion. We will calculate learning rates, discrimination (d'), and bias of each animal. Successful learning will be animals that achieve a d' of >1.75 (**Fig. 6b**). In pilot studies, we have previously successfully trained animals on this task when the target sound is a pure tone. Results will be analyzed with t-tests; power analyses and past work[28,36,61] indicate we need 5-10 mice per condition. We found this behavior requires auditory cortex; bilateral infusion of the GABA agonist muscimol reduced performance (**Fig. 6c**).

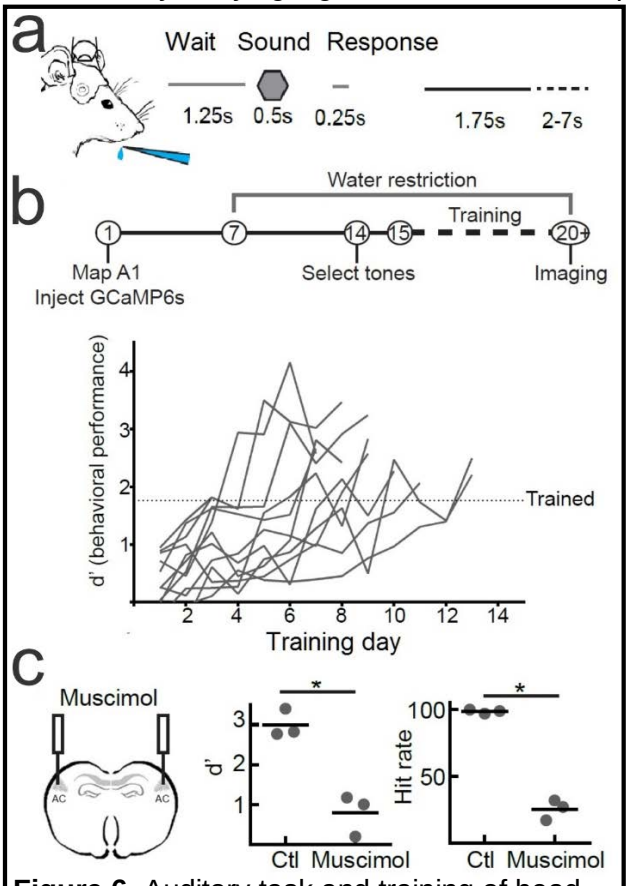

Figure 6. Auditory task and training of head-fixed mice. **a**, Mice lick to target sound for water reward. **b**, Training time; includes preparing mice for 2-photon imaging. Mice trained to criterion (d' > 1.75) in 3-10 days. **c**, Task requires auditory cortex; bilateral muscimol infusion reduced d' and hit rates.

For imaging, we will inject AAV1-SYN-GCAMP6s into AI for GCaMP6s expression[45,61,99]. Before training, we will implant headposts and cranial windows. Mice are acclimated to head fixation over 2 weeks for viral expression. We will perform 2-photon imaging of Ca^{2+} signals from head-fixed mice during training (**Fig. 7a-c**). Our data (**Fig. 7d**) show we can image the same cells over days in behaving mice. Fluorescence changes are quantified relative to background (dF/F) for each cell, with strict criteria for motion correction and artifact exclusion[45,61,100]. For excitatory cell imaging, we use *Thy1-GCaMP6* mice[101]. To selectively image interneurons, some C57Bl/6 mice are *PV-tdTomato*, *VIP-tdTomato*, or *SST-tdTomato*[45,61,102-104] (**Fig. 8**). A postdoc in the lab Kishore Kuchibhotla (key personnel) has extensive experience with 2-photon imaging[45,61,105-107]. Stimuli will be selected based on local AI frequencies. For tones, targets and foils will be within one octave; this will be starting frequency for FM sweeps with downsweeps for target, upsweeps for foil. In the animal shown in **Figure 7a**, median best frequency was 6 kHz; 8 kHz was chosen as target and 4.7 kHz as foil (**Fig. 7b**). Background noise from the 2-photon is ~40 dB SPL; stimuli are played at 70-80 dB SPL.

Experiments: We will perform six studies in behaving mice to show how auditory training refines AI.

1) We will image AI responses in untrained vs. fully-trained mice, comparing responses to targets and foils from the same cells during behavior ('active context') vs. passively during head-fixation without licktube ('passive context'). We hypothesize that AI responses differ in trained mice in active vs passive contexts. We expect that after switching to the active context (simply by adding the licktube), most neurons will be suppressed, but a sub-ensemble will have enhanced responses to targets and foils. This is supported by our pilot data: many cells respond to the target in the passive context, but decreased responses in the active context (**Fig. 8**). This task-engaged suppression replicates recent studies of AI and other cortical areas[104,108-113]. We also observed a sub-network of active-preferring cells encoding targets and foils after learning (**Fig. 8**). Context-dependent responses were stimulus-specific and task-dependent, not due to reward expectation or delivery or from motor signals during licking, because of context-dependent changes to foil responses when trained mice do not lick.

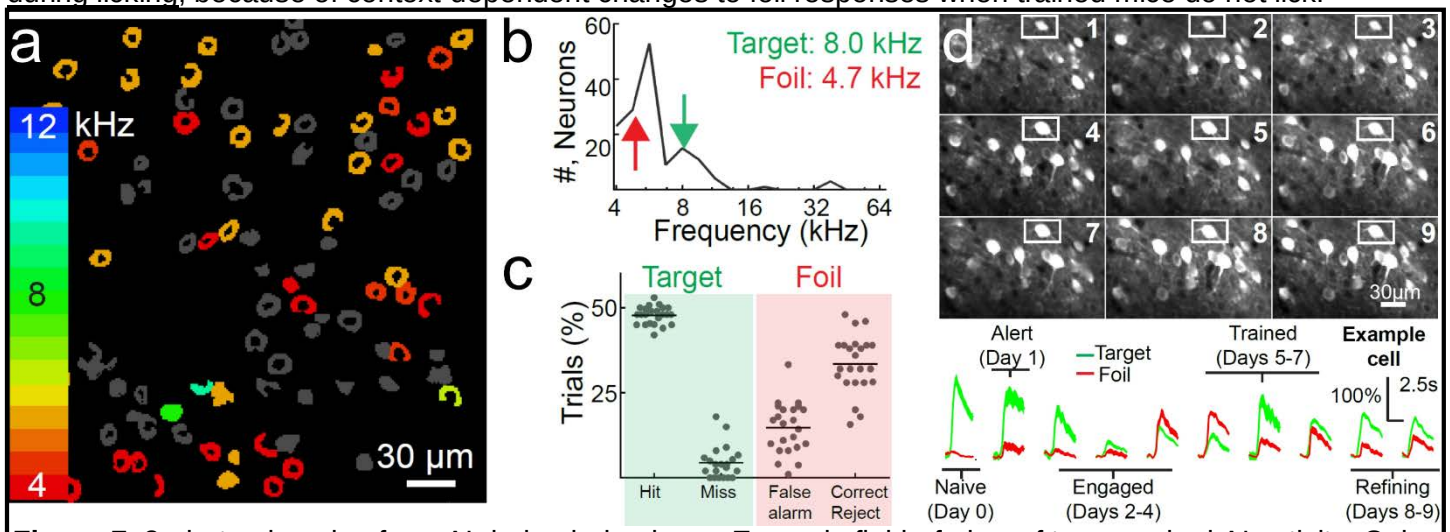

Figure 7. 2-photon imaging from AI during behavior. **a**, Example field-of-view of tone-evoked AI activity. Color, best frequency of each cell. Note low-kHz tuning. **b**, Best frequency histogram of all cells for this mouse. Target was 8 kHz, foil 4.7 kHz. **c**, Behavior of 23 mice. **d**, Imaging from same cell over nine training days.

2) We will image longitudinally from the same cells in each mouse over the entire training duration (**Fig. 7d**). We hypothesize that we can observe construction of the 'active ensemble' over days. Specifically, many cells will be first enhanced during day 1 in the active context, but as mice improve in performance (and learn to suppress licking to foils), the majority of cells become suppressed (compare day 0 and days 8-9, **Fig. 7d**).

3) We will image AI inhibitory cells, examining PV+, VIP+, and SST+ interneurons[45,61,102-104] in different mice during training. Our pilot data show that inhibitory cells are generally enhanced during behavior in trained mice (n=19/31 or 61% PV+, 12/20 or 60% SST+ cells significantly *increased* activity, compared to only 8/36 or 22% VIP+ and 142/620 or 33% excitatory cells that increased activity; **Fig. 8**). We predict PV+ and SST+ cells are 'first-responders' when training begins.

4) We will examine how stimuli statistics affect AI responses and behavior. We will compare mice trained to detect/recognize FM sweeps vs. tones, asking if this produces differential dynamics by which excitatory or inhibitory cells are modified by training. After reaching criterion, we will adjust stimuli to make the task more challenging (e.g., foils more spectrally similar to target).

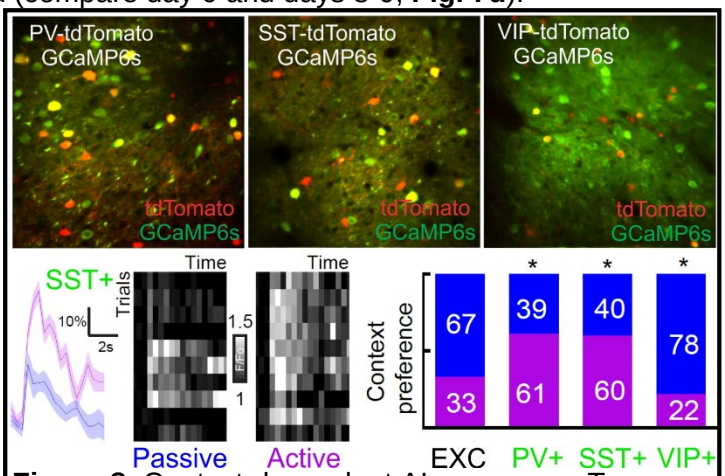

Figure 8. Context-dependent AI responses. Top, imaging from all three cortical interneuron types. Bottom, % of cells activated during behavior in trained mice (purple), vs suppressed (blue).

5) We showed that excitatory and inhibitory plasticity in mouse AI requires NMDA receptors[44]. We will ask if AI infusion of APV (NMDA receptor blocker, 0.1 mM) slows learning when given over days during training, or impairs performance when given to highly-trained animals.

6) We will relate single-trial performance of AI network activity to behavior, asking if AI responses differ on correct vs error trials. We are collaborating with Ken Miller (see letter of support) to build a single-trial decoder based on AI excitatory and inhibitory responses, both in terms of single neurons as well as population dynamics.

Discussion/Alternatives: Many studies have highlighted state-dependent responses[4,104,108-118], especially in AI. Here we ask how such state-dependent processing emerges with training. To understand how AI plasticity relates to auditory learning, it is vital to record from cell populations during behavior. Imaging is advantageous as we can track the same cells over days, clearly separating inhibitory from excitatory cells, and simultaneously image dozens of cells per trial. This enables us to try single-trial decoding at individual cell and network levels. If dF/F signals have poor temporal resolution for decoding, we will use deconvolution[119] to predict single spikes. If learning rates in initially-naïve mice are slow, we will try 'reversal learning'[28], first training mice on one target before switching to target and foils represented in the imaging window. Our pilot data and past studies[45,61,120-124] indicate that background noise from the microscope does not preclude imaging auditory-evoked responses, but if needed we will use insertable speakers and earplugs to reduce noise and effectively control stimuli. If changes to AI lag or are uncoupled from behavioral improvement, we will image from cortical 'belt' areas around AI[125].

Subaim 1b. To examine plasticity of AI excitatory and inhibitory synaptic inputs during auditory training.

Next we examine the synaptic basis for changes to AI spiking responses, by making whole-cell recordings in behaving mice. We hypothesize that inhibitory inputs are more context-dependent than excitatory inputs. There are two co-existing phenomena we will try to explain: that training leads to stable long-term enhancement of AI representations of task-relevant stimuli[3-11], but also that during behavior, most AI cells are suppressed[108-113].

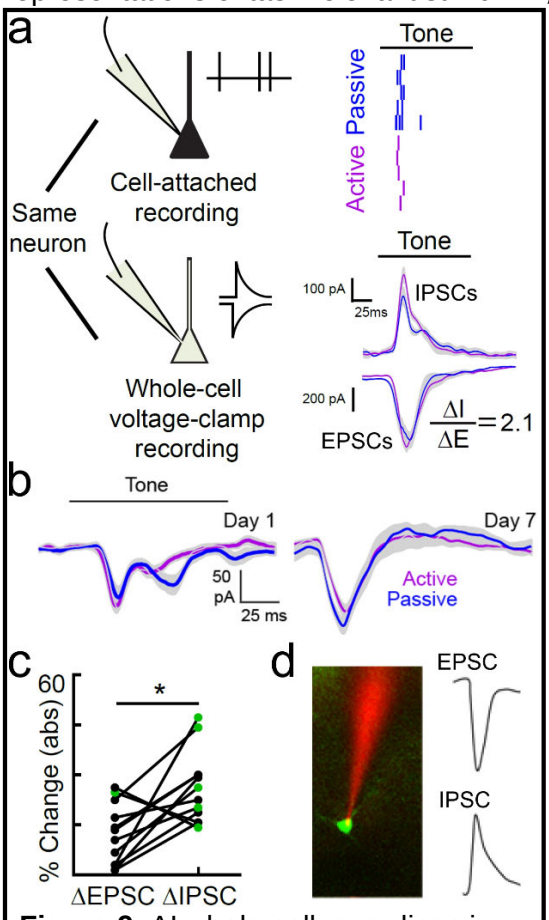

Figure 9. AI whole-cell recordings in behaving mice. **a**, Recording spikes & currents from same cell during active behavior vs passive state. **b**, Two cells recorded in same mouse on days 1, 7 of training. **c**, Active context modulates IPSCs more than EPSCs. **d**, Targeted recording of SST+ interneuron in vivo.

Methods: In mice from Subaim 1a, we will drill holes through the coverslip for whole-cell voltage-clamp recording during behavior. We will measure target- and foil-evoked excitatory/inhibitory postsynaptic currents (E/IPSCs) by holding cells at different potentials (**Fig. 9a**). To monitor synaptic changes over multiple cells and days, we take advantage of co-tuning of E/IPSCs, computing IPSC/EPSC ratios for single stimuli or E:I correlation over frequencies, reliable measures of hours-long changes in AI. Analysis will be with ANOVAs or Mann-Whitney tests. In vivo whole-cell recordings are lower-yield than imaging, but from power analyses and our past studies[28,36,41,42,62], we expect to need ~30-50 cells per study, feasible in this project timeline.

Experiments: We will perform four studies of AI synaptic changes.

1) We will record from neurons over different days of training, asking how EPSCs and IPSCs are modified by auditory training. We can record from different cells in the same animal over days (**Fig. 9b**). In our pilot data, voltage-clamp recordings from trained mice during behavior showed that IPSCs were modulated by behavioral context more than EPSCs; some cells had stronger tone-evoked inhibition during the active behavioral context vs passive (**Fig. 9a**; ΔI/ΔE=2.1), and other cells have less inhibition relative to excitation in the active context. We predict that EPSCs and IPSCs evoked by targets and foils are first enhanced during behavior (via noradrenergic lowering of inhibitory tone, to be examined in **Aim 2**), together with increased hits and false alarms. However, behavioral improvement (by reducing false alarms) over days is paralleled by AI inhibitory plasticity, leaving EPSCs generally intact but leading to learned context-dependent modulation of inhibition (via acetylcholine, also tested in **Aim 2**). Specifically, we hypothesize that most cells have higher IPSC/EPSC ratios during behavior following training, while a fraction (the active ensemble) will have much lower I/E ratios (**Fig. 9c**).

2) In some cells, we will first measure spiking in cell-attached mode before break-in. We use simulations to predict spikes from synaptic input[41,42,62], relating synaptic to spiking changes in Subaim 1a.

3) We will make 2-photon targeted recordings from excitatory or inhibitory cells (**Fig. 9d**), from cells modulated by context over learning. We predict decreased I/E ratios in PV+ and SST+ cells, increasing activity during behavior (**Fig. 8**), but VIP+ cells are suppressed due to high I/E ratios.

4) Imaging experiments in Subaim 1a necessarily focus on superficial layers. With cell-attached and whole-cell recordings, we will record from cells from each cortical layer, asking if cells across lamina show similar patterns of context-dependent modulation (in highly-trained animals) and are modified over learning at similar rates.

Discussion/Alternatives: Here we monitor synaptic plasticity during auditory learning. Successful completion of these experiments will provide new data directly relating AI changes to learning and behavioral performance, in real time as both neural and behavioral changes occur. We will carefully examine synaptic and spiking responses during early phases of learning, asking if there are intracellular correlates of 'eureka' moments (e.g., bursts or NMDA spikes)[10,126-132]. If targeted recordings or recordings in mice for imaging in Subaim 1a are too low-yield, we will make blind whole-cell recordings, which we previously used to study synaptic plasticity in vivo[28,36,41,42].

Aim 2. To ask how cholinergic and noradrenergic modulation affect auditory learning and AI plasticity

What mechanisms convey behavioral context to AI, and how are these mechanisms recruited during learning? We will record from AI during behavior, using optogenetics and pharmacology to ask if and when the cholinergic vs noradrenergic systems are necessary for learning and peak performance (Subaim 2a). We will ask if pairing task-relevant sounds with cholinergic and/or noradrenergic modulation accelerates learning or enhances peak performance (Subaim 2b). We will determine synaptic mechanisms by which cholinergic and noradrenergic modulation enable AI plasticity during learning. Our pilot data (**Fig. 10**) show that atropine impairs context-dependent modulation of behavioral and neural responses in highly-trained mice, motivating these studies.

Subaim 2a. To examine when noradrenergic and cholinergic modulation of AI is required for auditory behavior. We will examine behavioral performance on the auditory task, using optogenetic and pharmacological methods to suppress noradrenergic or cholinergic neuromodulation at different phases of learning.

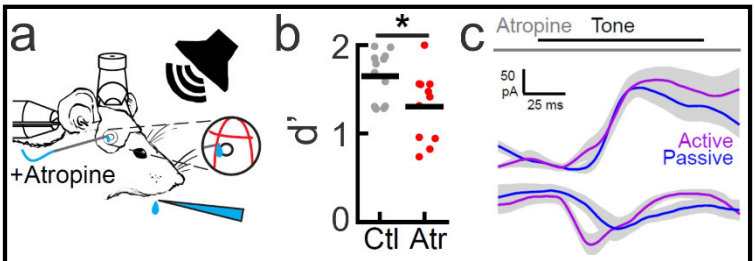

Figure 10. Atropine in AI during behavior. **a**, Setup. **b**, Reduced hit rate. **c**, No contextual modulation of IPSCs.

Methods: We will virally express halorhodopsin in nucleus basalis of *ChAT-Cre* mice[37,38,133] or locus coeruleus of *TH-Cre* or *DBH-Cre* mice[134-136], via stereotaxic injection of AAV1.EF1a.DIO.eNpHR3.0, and optical fibers implanted in these nuclei. We will inject/implant some wild-type mice. We will implant cannulas in some mice bilaterally in AI for local drug infusion. In some mice we image and record in vivo as in Subaims 1a,1b. These studies each require ~4-8 mice and cells, analyzed with t-tests or ANOVAs.

Experiments: We perform seven studies to ask if/when modulation is *necessary* for learning.

1) We will pharmacologically block noradrenergic receptors each day during training. We will first try α- and β-receptor blockers together. Previously we found that phentolamine (α-noradrenergic receptor antagonist, 0.1 mM) prevented AI plasticity after locus coeruleus pairing[28]. We will also try propranolol (β-receptor antagonist) in a few mice. As α_1 and α_2 have opposite effects on excitability depending on site of action[26,51,137], we will use progressively more selective receptor blockers to determine specific subtypes for learning and performing this task. We hypothesize that blocking noradrenergic receptors impairs performance only early in training rather than later. We may be able to assess blockade daily in the same mice, training them 2/day with a saline-infusion control session followed by drug to ask how noradrenergic blockade impacts behavior day-by-day.

2) AI atropine reduces behavior in highly-trained mice (**Fig. 10a,b**). We will ask if atropine (to block muscarinic receptors) or nicotinic blockade slows learning rate, impairing performance later rather than earlier in training. If needed, drugs will be given on only one day; but as above, we will also try drug infusion every day per mouse.

3) We will pharmacologically block noradrenergic and cholinergic receptors together. After determining temporal sensitivity and receptor identity (muscarinic vs nicotinic, α- vs β –noradrenergic), we will ask if noradrenergic and cholinergic systems act together linearly, if there are periods early or late in training when these systems act together synergistically (as in past studies of ocular dominance plasticity[47,85]), or if with both systems blocked there is still some residual performance (e.g., possibly supported by dopaminergic-based reinforcement).

4) We will perform 2-photon imaging of excitatory and inhibitory cell populations. Informed by results from the previous experiments in wild-type mice, we will block noradrenergic or cholinergic receptors during training, to measure effects on specific inhibitory cell populations. We will ask if there is temporal sensitivity in inhibitory responses (e.g., interneurons of each type are affected more by noradrenergic blockade early in training, but more impacted by cholinergic blockade later in training), and/or if there is cell-type specificity (e.g., PV+ cells are more affected by cholinergic antagonists while SST+ cells more affected by noradrenergic antagonists). Some cell-attached and whole-cell recordings will be made to confirm imaging results at the intracellular level.

5) We will optogenetically suppress activity in locus coeruleus noradrenergic neurons during training. We predict that silencing locus coeruleus on the first day of training will completely prevent learning, as animals will recognize the presence of the context cue (i.e., licktube). Silencing on days 1-3 will also slow learning rates.

However, later in training we hypothesize that locus coeruleus inactivation is less deleterious. Specifically, we predict that as false alarm rate to foils begins to decrease, locus coeruleus norepinephrine is no longer needed.

6) We will optogenetically suppress activity in nucleus basalis cholinergic neurons. In contrast to locus coeruleus, we hypothesize that early in training (e.g., day 1) the cholinergic system is dispensable. However, the effects of optogenetic suppression will be greater with each subsequent day of training, especially for peak performance.

7) We will image AI cell populations during episodes of optogenetic silencing. We will compare optogenetic suppression to pharmacology, to control for potential issues related to lack of specificity or drug delivery in vivo.

Discussion/Alternatives: We hypothesize that locus coeruleus controls the initial increase in performance from zero in untrained animals due to increased licking (hits and false alarms), while nucleus basalis is involved in AI inhibitory control, maintaining hit rate and reducing false alarms over days. Previous studies and our own work[13,23-26,28,36-40,63-65] indicate that cholinergic and noradrenergic systems are important for sensory tasks such as the auditory behavior used here. However, there may be other mechanisms we need to consider, including nicotinic receptor control of auditory thalamocortical excitability[91]. We are using a reward-based paradigm, and so dopaminergic modulation also might be important as suggested by past studies[75-77]. If needed we will examine the VTA dopamine system, or modify our task to use aversive reinforcement as in other studies of AI[4,138,139]. We have successfully used 0.1 mM doses to block effects of nucleus basalis and locus coeruleus stimulation[28,36], but if needed we will measure dose-response curves or cellular mechanisms in mouse AI brain slices as we have done previously[43]. There are many opto- and pharmacogenetic compounds now available; if halorhodopsin expression is poor or does not provide sufficient silencing, we will try archaerhodopsin or DREADD-based inactivations, as we have done previously[58]. We will perform calibration experiments in vivo (and in brain slices if needed) to ensure that optical stimulation effectively hyperpolarizes neurons to prevent them from firing.

Subaim 2b. To ask how norepinephrine and acetylcholine separately and synergistically affect learning rate.
We will optogenetically stimulate the noradrenergic system of the locus coeruleus and/or the cholinergic system of the nucleus basalis, pairing optical release of endogenous neuromodulation with auditory stimuli. These studies directly extend our past work showing that neuromodulatory pairing leads to long-lasting improvements in rat auditory detection and recognition (**Figs. 4,5**); moreover, one episode of locus coeruleus pairing on the first day of stimulus reversal was found to accelerate learning rate and enhanced behavior days later (**Fig. 11**).

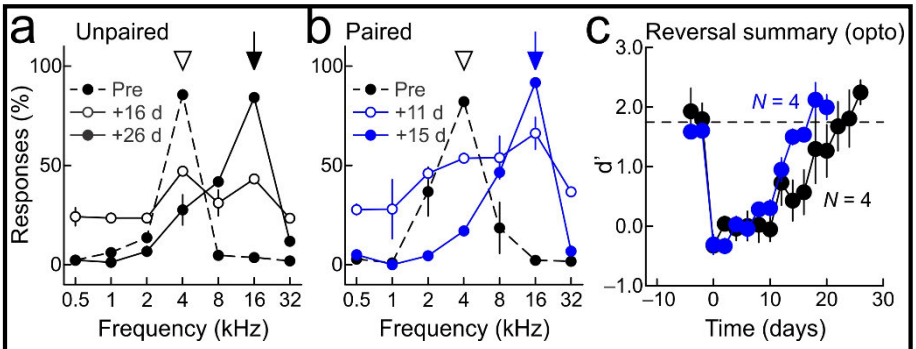

Figure 11. One pairing episode (5 min on day 0) of optogenetic locus coeruleus stimulation accelerated reversal learning. **a**, Unpaired animal, switching target on day 0 from 4 kHz (arrowhead) to new 16 kHz target. **b**, Paired animal. **c**, Summary (ref. 28).

Methods: Methods are similar to Subaim 2a, but we express channelrhodopsin-2[28,42,43,140] 'ChETA' variant in nucleus basalis of *ChAT-Cre* or locus coeruleus of *TH-Cre* or *DBH-Cre* mice, via stereotaxic injection of pAAV5-Ef1a-DIO-ChETA-EYFP. Some animals will have optical fibers implanted in nucleus basalis or locus coeruleus, other animals have fibers bilaterally implanted in AI.

Experiments: We will perform six studies to determine how AI modulation can improve learning and affect behavior.

1) We will pair target sounds with locus coeruleus stimulation during training. Some animals will be optically stimulated in locus coeruleus, other animals will receive optical stimulation only in AI; we expect results to be similar. Some animals will receive only a single episode of pairing (5 min, sound concurrent with 20 Hz optical stimulation; **Fig. 11**), other mice will receive stimulation on every training session. We hypothesize that learning will be accelerated by a single episode of pairing early in training; repetitive locus coeruleus stimulation may instead impair performance[28,141].

2) We will pair target sound presentation with nucleus basalis stimulation during training. As above, some animals will receive a single episode of pairing; other mice will receive daily pairing. We hypothesize that a single episode will not have lasting gains in performance; this is supported by our past studies of nucleus basalis pairing and auditory behavior in rats (**Fig. 4b,d**). However, we expect that multiple rounds of pairing will lead to lasting gains.

3) We will pair targets with locus coeruleus and nucleus basalis stimulation together. We will express ChETA both in locus coeruleus and nucleus basalis in the same animals (with a non-floxed ChETA via synapsin promoter). Although we lose neurochemical specificity for one modulator, this provides information as to whether effects of pairing add linearly, are synergistic, or occlude each other depending on when pairing is performed.

4) We will image and record during training and optogenetic stimulation of the locus coeruleus and/or nucleus basalis. This can be done in many of the same animals used above in experiments 1-3. We have two goals here- first, to see if optogenetic stimulation accelerates or disrupts changes in IPSC/ESPC ratios expected to emerge over training in these stimulated animals. Second, to record during pairing target sounds with nucleus basalis and/or locus coeruleus stimulation, to ask how optogenetic neuromodulatory release affects EPSCs, IPSCs, and spiking in excitatory or inhibitory cells in awake animals. As we hypothesize that modulatory effects will be similar as we previously described under anesthesia (**Fig. 2**), this reduces the total number of experiments required.

5) We will pair non-targets with locus coeruleus or nucleus basalis stimulation. We predict that off-target pairing with locus coeruleus stimulation impairs performance, while pairing foils with nucleus basalis stimulation may instead accelerate learning (as also predicted for target pairing).

6) We will examine effects of unpaired stimulation, asking if activation of either modulator system can lead to behavioral responses outside of behavioral context (i.e., when stimuli are passively presented in absence of licktube). Our data show that highly-trained mice lick after nucleus basalis stimulation (**Fig. 12**).

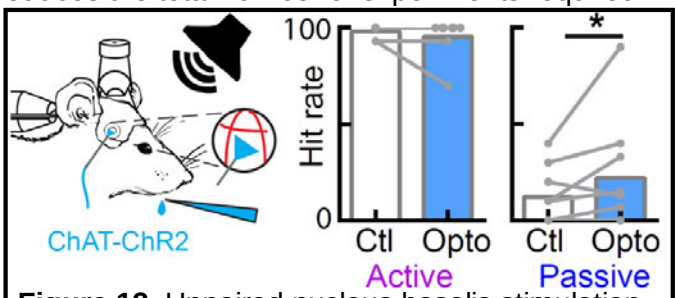

Figure 12. Unpaired nucleus basalis stimulation in trained mice can induce licking without licktube (behavioral context) if tone is passively played.

Discussion/Alternatives: While studies of Subaim 2a ask about the necessity of neuromodulation for perceptual learning and task performance, here we ask if stimulation of these systems can enhance learning or suffice to provide behavioral context. If viral expression is problematic, we can try using *ChAT-ChR2* mice, expressing channelrhodposin-2 in cholinergic neurons. While these animals are reported to have some behavioral problems[142], we found on our task they perform similar to wild-types (**Fig. 12**). Past studies have found good correspondence between AI plasticity induced by pairing sounds (generally pure tones) with neuromodulator agonists and stimulation (generally electrical) of modulatory centers[1,2,10,28,35,42,43,138]. If initial learning is complicated by procedural aspects of the task (learning to head-fix, lick for reward) and confounds these studies, we will study reversal learning in trained mice, with tone and foil stimuli reversed as we did previously (**Fig. 11**).

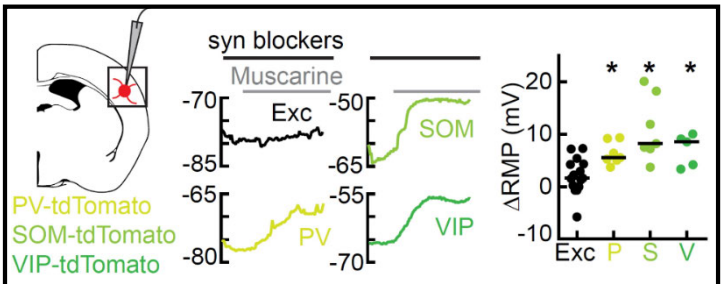

Figure 13. Muscarine depolarizes AI interneurons significantly more than AI excitatory cells.

Our goal is to provide mechanistic insights for changes in behavioral performance, as related to AI excitatory and inhibitory inputs (via voltage-clamp recordings) and spiking output (via 2-photon imaging and cell-attached recording). Our data show that muscarine in mouse AI depolarizes all cells, but inhibitory cells more than excitatory cells (**Fig. 13**). Consistent with work from other labs[33,138,143], neuromodulation depolarizes cortical interneurons to increase spontaneous GABA release and IPSC frequency, depleting the available pool of GABA available. Alternatively, modulation might decrease transmitter release directly[144]. In either case, stimulus-evoked IPSC amplitude is reduced (**Fig. 2**).

These interactions between multiple cell types lead to complex AI network dynamics on single trials. We are collaborating with Ken Miller (see letter of support) to build network models that describe these dynamics in terms of the lower-level cellular responses. We are also collaborating with Susan Sara, world expert on locus coeruleus, cortical responses, and behavioral control; Dr. Sara has an office at NYU and has been helping us target locus coeruleus for recordings and injections (see letter of support), to help ensure success of this project.

Aim 3. To examine when sensory stimuli and behavioral context activate modulatory systems

What aspects of training and behavioral context activate modulatory systems and promote AI plasticity? We will ask if there is a sequence of AI modulation over task learning; specifically if the noradrenergic system is recruited transiently during the first day, while the cholinergic system comes on-line over subsequent days and is needed for steady-state performance. We will record from locus coeruleus and nucleus basalis, and perform 2-photon imaging of modulatory axons within AI in trained animals and over the duration of learning. Recently we found that tone-evoked responses with short latency are induced in locus coeruleus neurons after pairing tones with locus coeruleus stimulation[28]. Here we ask if plasticity in nucleus basalis and/or locus coeruleus is important for learning or task performance. We test the hypothesis that the noradrenergic system is initially active early in training (e.g., on day 1), while cholinergic activity increases gradually over days in parallel with performance. We note that the studies in **Aim 3** do not depend strongly on particular findings or details of studies in **Aims 1** or **2**.

Methods and Experiments: We will use five approaches to measure the responses of modulatory neurons in behaving mice. As shown by our preliminary data (**Figs. 5,14,15**), each of these techniques- in vivo axonal imaging, op-tetrode recording, fiber photometry, and whole-cell recordings- are working in the lab.

1a) We will image cholinergic axons from nucleus basalis projecting within AI during behavior. 2-photon imaging will be performed as in Subaim 1a, but we will express GCaMP6s in cholinergic neurons via stereotaxic nucleus basalis injection of AAV1-SYN-GCAMP6s in *ChAT-Cre* mice. After ~2 weeks for expression, we will image Ca^{2+} signals in cholinergic axons in AI. Our pilot data (**Fig. 14a-c**) and previous results demonstrate feasiblility[61,145]; we will be careful to correct for motion artifacts. We will play tones and FM sweeps of varying frequencies and intensities, measuring axonal Ca^{2+} signals both during auditory training and passive presentation. Our pilot results show that in highly-trained animals, cholinergic axons are much more responsive during correct trials than during passive presentation (**Fig. 14a-c**). We will monitor the same axons over the course of training to determine when responses occur, and if axonal responses are also evoked by task variables such as licktube insertion. We hypothesize that cholinergic axon activation gradually increases over learning and is required for successful task performance in experienced animals. For these studies we expect to need ~5-8 animals.

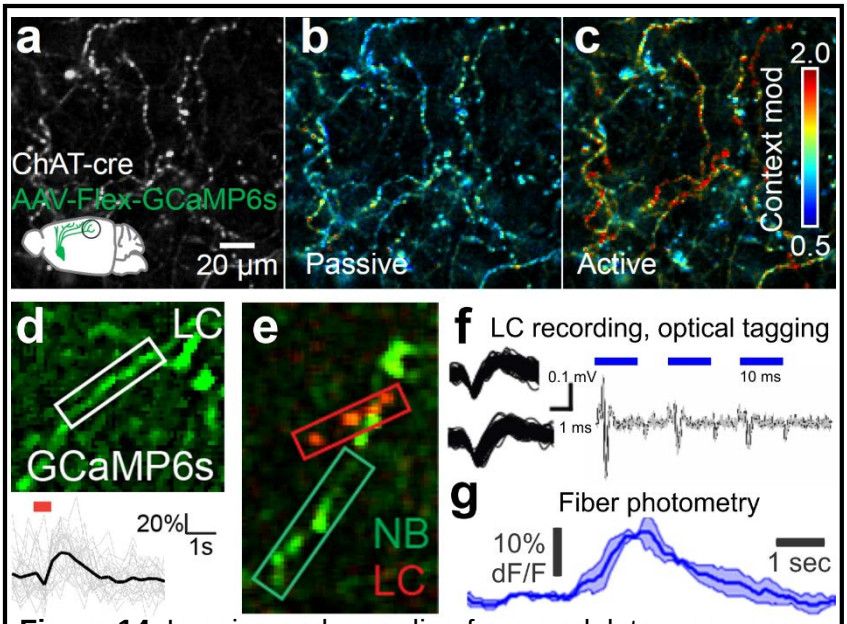

Figure 14. Imaging and recording from modulatory neurons. **a-c,** In vivo 2-photon imaging of GCaMP6s in cholinergic axons of behaving mouse AI. Axonal responses are strongly enhanced by behavioral context. **d**, In vivo imaging of LC axons within AI; bottom, sound-evoked Ca^{2+} responses. **e**, Two-color imaging in AI with RCaMP in LC and GCaMP6s in NB. **f**, Multi-tetrode recording in vivo from optically-tagged LC noradrenergic units. **g**, In vivo fiber photometry in LC.

1b) We will image from locus coeruleus noradrenergic axons in AI, injecting AAV for GCaMP6s expression in *TH-Cre* or *DBH-Cre* mice. Our pilot data in a naïve untrained animal shows large locus coeruleus axon responses in AI to surprising sounds (**Fig. 14d**); we expect these responses to diminish with exposure or experience.

1c) We will perform simultaneous imaging of cholinergic and noradrenergic axons within AI of the same animal during behavior (**Fig. 14e**), to directly compare responses of modulatory fibers over days as mice learn the task. GCaMP6s will be expressed in one area, the red-shifted indicator RCaMP[146] in the other.

2) We will use multi-optrode array recording, implanting electrodes with fibers for optical stimulation[147] in nucleus basalis of *ChAT-Cre* or *ChAT-ChR2* mice with channelrhodopsin-2 in cholinergic neurons. In other *TH-Cre* or *DBH-Cre* mice we will implant op-tetrodes in locus coeruleus. We will identify specific modulatory cells by optical stimulation[148]; and analysis done on light-sensitive units (**Fig. 14f**), which are cholinergic or noradrenergic cells tagged by opsin expression. Histological methods will verify ChETA expression and electrode placement. We will compare local modulatory signals in AI (measured axonally in studies 1a-c) with activation of more global signals by recording from cell bodies. We will ask if modulatory neurons respond to task variables, and if stimulus specificity changes during auditory training.

3) We will perform fiber photometry in nucleus basalis or locus coeruleus of transgenic mice expressing GCaMP6s only in modulatory neurons. Fiber photometry[149] is essentially an 'optical LFP', measuring bulk GCaMP6s signal representing integrated firing of GCaMP6s-expressing cells. Although this decreases our temporal resolution somewhat and we lose single-unit specificity, photometry signals are straightforward to measure and quite stable. We will compare photometry signals (**Fig. 14g**) to axonal responses in AI (experiment 1) and single-units in experiment 2. If modulatory projections to AI are topographic and it proves challenging to obtain single-unit tetrode recordings from neurons activated during behavior, photometry may provide suitable data on the bulk response of cholinergic vs noradrenergic cells to behavioral context early vs late in training.

4) We will perform in vivo whole-cell recordings from nucleus basalis or locus coeruleus neurons. We have successfully obtained whole-cell recordings from rat locus coeruleus in vivo (**Fig. 5a,b**). In our previous study, we found that about half of all locus coeruleus cells developed auditory responses after conditioning or pairing[28]. Whole-cell pipettes contain biocytin and we will recover cells histologically, co-staining for TH or ChAT to

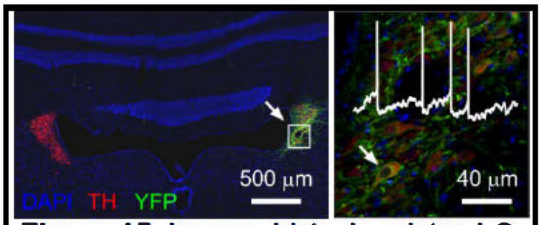

Figure 15. Immunohistochemistry: LC whole-cell recording and biocytin fill.

determine cell identity (**Fig. 15**). While it will be challenging to find responsive nucleus basalis cells, this is a 'high-risk, high-reward' experiment, not critical to other goals of this project, and even a few cells (~4-5 required for statistical power) may be informative. We are routinely able to obtain ~5-10 recordings per session, including cell-attached recordings, allowing us to screen many cells.

5) Finally, we aim to determine if these changes in modulatory neural responses are due to local plasticity in subcortical areas, and if preventing this plasticity affects learning. Modulatory neurons are activated by task context, and develop responses to conditioned stimuli after training[23,24,28]. Emergence of auditory responses in nucleus basalis or locus coeruleus (with short latency and present even under anesthesia; **Fig. 5**) are potentially essential components by which previously-innocuous stimuli develop meaning, and thus it is critical to understand the mechanisms and behavioral relevance of these changes for auditory learning. Here we perform two studies, asking first if infusion of APV (to block NMDA receptors) or modulatory receptor antagonists prevent auditory responses or responses to context cues (e.g., licktube) from arising in these nuclei. We will then infuse these blockers into either locus coeruleus or nucleus basalis on the first day of training (in some animals) or each day of training (in other animals) to determine how behavioral performance and learning is impacted when modulatory plasticity is blocked.

Discussion/Alternatives: We aim to obtain a multi-dimensional view of how behavioral context and task variables differentially engage cholinergic vs. noradrenergic modulatory systems. This entails examining subthreshold vs suprathreshold responses, and local vs more global signals over training. We hypothesize that the presence of the licktube (which signals active behavioral context) initially activates locus coeruleus, before these neurons begin responding to task-relevant sounds (both targets and foils; **Fig. 14d,g**). As performance improves, locus coeruleus activation sharply declines, and instead cholinergic neurons begin responding (shown by our preliminary data in **Fig. 14a-c**). We will examine the behavioral importance of the emergence of these responses. Our past work (**Fig. 5**) showed that NMDA receptors were critical for this plasticity, but we will examine other candidate mechanisms (e.g., modulatory autoreceptors) if necessary. In future studies beyond the scope here, we will map the functional anatomy that provides potential auditory information to modulatory centers.

These are technically-challenging methods, but we have experience and success with each approach. Past studies showed that surprising events or contextual cues increase cholinergic or noradrenergic tone, often measured with amperometry in rodent and primate cortex[64,65,150]. However, this is indirect with low spatiotemporal resolution, making it difficult to relate degree of modulatory activation with single-trial neural or behavioral responses. Instead, here we take advantage of mouse lines for modulatory control, for optical identification of cell-types (**Fig. 14**). This is important, as locus coeruleus to some degree and nucleus basalis to a potentially high degree are neurochemically heterogeneous[29,39,65]. In vivo whole-cell recordings might reveal if even in naïve (untrained) animals, these neurons receive sensory-evoked synaptic inputs, but these inputs might be subthreshold or masked by inhibition to keep modulatory cells from spiking. It may be technically infeasible to make in vivo whole-cell recordings from cholinergic cells given their sparsity; we and others have already made in vivo whole-cell recordings from locus coeruleus noradrenergic neurons[28,151]. Conversely, it may be difficult to perform tetrode recording from locus coeruleus. For these reasons we use multiple methods to ask the same questions, and provide new data on spatial and temporal patterns of AI neuromodulation during auditory training.

Summary: Neuromodulation is important for auditory plasticity and perceptual learning. However, it is unclear how two of the main modulators involved in these processes- acetylcholine and norepinephrine- are naturally recruited during auditory training to differentially modulate target circuits. Our studies will provide new insight into these mechanisms, reveal how cortical plasticity is involved in auditory behavior, and suggest how modulatory systems are activated and modified by training for effective control of auditory perception. In **Aim 1**, we ask how auditory training relates to AI plasticity measured in behaving mice. We perform *in vivo* whole-cell recordings and imaging to determine dynamics of response changes. In **Aim 2**, we examine distinctions between cholinergic and noradrenergic modulation at different phases of learning. In **Aim 3**, we record from these systems and ask if they are plastic, relating auditory responses in modulatory axons and neurons to auditory perceptual learning.

AIMS	YR 1	YR 2	YR 3	YR 4	YR 5
1a. AI imaging during behavior	KK,EM,RCF				
1b. In vivo whole-cell recordings	KK,NL,RCF				
2a. Necessity of AI neuromod	KK,NL,RCF				
2b. Does AI mod aid learning?		KK,NL			
3. Plasticity of neuromodulation		KK,NL, EM,RCF			

Feasibility: All Aims are feasible in 5 yrs. [redacted] or successors thereafter.

Progress Report Publication List

For the original R01 proposal, we published several papers related to rodent auditory perception and cortical plasticity, including one major study (Martins and Froemke, Nature Neuroscience 2015) that constitutes Aim 1a, 1b, and almost all of the proposed studies in Aims 2 and 3. We are examining loci of plasticity both in vitro and in vivo (as relates to Aim 1c). We have two manuscripts that were favorably reviewed, now being revised for resubmission, involving recordings in rat primary auditory cortex, frontal cortex, and locus coeruleus during the auditory behavior used in this application (Carcea et al., Nature Communications under revisions; Insanally et al., Nature under revisions).

Froemke RC, Carcea I, Barker AJ, Yuan K, Seybold B, Martins ARO, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE. Long-term modification of cortical synapses improves sensory perception. Nature Neuroscience 2013; 16:79-88. PMC: 3711827

Carcea I, Froemke RC. Cortical plasticity, excitatory-inhibitory balance, and sensory perception. Progress in Brain Research 2013; 207, 65-90. PMID: 24309251 PMC: 4300113

Martins ARO, Froemke RC. Coordinated forms of noradrenergic plasticity in the locus coeruleus and primary auditory cortex. Nature Neuroscience 2015; 18:1483-1492. PMID:26301326 PMC: 4583810

Marlin BJ, Mitre M, D'amour JA, Chao MV, Froemke RC. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 2015; 520:499-504. PMID: 25874674 PMC: 4409554

D'amour JA, Froemke RC. Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. Neuron 2015; 86; 514-528. PMID: 25843405 PMC: 4409545

Ponte Costa R, Froemke RC, Sjöström PJ, van Rossum MC. Unified pre- and postsynaptic long-term plasticity enables reliable and flexible learning. eLife 2015; 4. PMID: 26308579 PMC: 4584257

Froemke RC. Plasticity of cortical excitatory-inhibitory balance. Annual Review of Neuroscience 2015; 38, 195-219. PMID: 25897875 PMC: 4652600

Froemke RC, Schreiner CE. Synaptic plasticity as a cortical coding scheme. Current Opinion in Neurobiology 2015; 35, 185-199. PMID: 26497430 PMC: 4641776

King J, Insanally M, Jin M, Martins ARO, D'amour JA, Froemke RC. Rodent auditory perception: critical band limitations and plasticity. Neuroscience 2015; 296, 55-65. PMID:25827498 PMC: 4426073

Mitre M, Marlin BJ, Schiavo JK, Morina E, Norden S, Hackett TA, Aoki C, Chao MV, Froemke RC. A distributed network for social cognition enriched for oxytocin receptors. Journal of Neuroscience 2016; 36:2517-2535. PMID: 26911697 PMC: 4764667

Insanally M, Trumpis M, Wang C, Chiang CH, Woods V, Bossi S, Froemke RC, Viventi J. A low-cost, multiplexed μECoG system for long-term, reliable high-density recordings in rodents. Journal of Neural Engineering 2016; 13:026030. PMID: 26975462, PMCID: in process.

King J, Shehu I, Roland JT, Svirsky MA, Froemke RC. A physiological and behavioral system for hearing restoration with cochlear implants. Journal of Neurophysiology 2016; in press.

Cohen S, Ma H, Kuchibhotla K, Watson BO, Buzsáki G, Froemke RC, Tsien RW. Excitation-transcription coupling in parvalbumin-positive interneurons employs a novel CaM Kinase-dependent pathway distinct from excitatory neurons. Neuron 2016; 90:292-307. PMID: 27041500 PMC: 4866871

Animal Subjects Justification

1. Proposed Use of Animals: All experimental procedures used in this study are currently approved under an NYU School of Medicine IACUC protocol: "Synaptic Plasticity in Rodents", protocol number 160611-01, approved on 6/13/2016.

Mice: Experiments in mice are necessary for these proposed studies. For most experiments, we will use adult male and female C57Bl/6 mice 6-12 weeks old and weighing 20-30 g. We have verified that our animals hear at these ages, and our experiments are not compromised by hearing loss. Some animals will be *ChAT-Cre* mice expressing Cre recombinase in cholinergic neurons (ChAT: choline acetyltransferase), other animals will be *TH-Cre* or *DBH-Cre* mice expressing Cre recombinase in noradrenergic neurons (TH: tyrosine hydroxylase; DBH: dopamine-β-hydroxylase). In a few experiments we will use *ChAT-ChR2* mice expressing channelrhodopsin-2 in cholinergic neurons. For imaging inhibitory neurons in *PV-tdTomato*, *SST-tdTomato*, and *VIP-tdTomato* mice, *PV-Cre*, *SST-Cre* and *VIP-Cre* animals have been crossed with the *Ai9 tdTomato-flox* reporter line (Allen Institute). We will also use *Thy1-GCaMP6* mice for imaging excitatory neurons. As shown by our preliminary data, we have already obtained all of these mouse lines and are actively maintaining breeding colonies at NYU.

For optogenetic control of cholinergic or noradrenergic neurons, we will perform stereotaxic viral injections in *ChAT-Cre*, *TH-Cre*, or *DBH-Cre* mice, similar to our previous studies in mice and rats[28,42,43,45,58-61]. Mice will be anesthetized with 0.7-2.5% isoflurane, placed into a stereotaxic apparatus, and a craniotomy performed over nucleus basalis (from bregma: 0.5 mm posterior, 1.85 mm lateral, 4.5 mm ventral) or locus coeruleus (from bregma: 5.5 mm posterior, 0.85 mm lateral, 3.65 mm ventral). Injections will be performed with a 5 μL Hamilton syringe and a 33 gauge needle. Cre-inducible pAAV5-EF1α:-DIO-ChETA-EYFP virus53 will be injected at 0.1 nl/s for a final injection volume of 1.2-1.5 μl. An optical fiber ferrule will be implanted either subcortically or in electrophysiologically-identified AI (100 μm ventral). Some wild-type animals will be cannulated in a similar manner for drug delivery. The craniotomy and implant will be sealed with dental cement, and the virus given a minimum of two weeks to express.

Overall, we plan on using approximately ~295-540 animals for the experiments described in this proposal (see Section 2 below for breakdown of animal use by specific Aims).

Behavioral training: All behavioral events (imaging time-stamping, stimulus delivery, water delivery and lick detection) will be monitored and controlled by custom-written programs in MATLAB interfacing with an RZ6 auditory processor (Tucker-Davis Technologies). After at least 7 days of water restriction, training would commence. Training takes place during the day and begins with habituation to head-fixation, immediately followed by water-sampling sessions. Mice typically learn to lick for 3-5 μl droplets of water within 1-2 sessions. Once the animals lick reliably, they are placed in the complete behavioral task with minimal shaping. We utilize a go/no-go behavior task with the target and foil chosen based on the two-photon targeted imaging. A behavioral block typically consists of 100 trials and with 4 blocks of training per day (total of ~400 trials per day). Target versus foil trials were pseudo-randomly ordered, each of which consisted of scan onset (imaging), pre-stimulus period (1.25 s), stimulus period (100-500 ms), delay (250 ms), a response period (1.75 s), and an inter-trial interval (ITI with variable duration as described below). Mice only receive water for correct responses to the target during the response period. Incorrect licks after the foil presentation (a false alarm) are mildly punished by increasing the ITI. Animals are not punished if they licked during any other time epoch (i.e., if animal licks in the pre-stimulus period, tone presentation or delay period). This enables us to confirm that animals actively increase lick rate for targets during hit trials and reduce lick rate for foil tones during correct rejects (from baseline lick rate to zero during response window). This measurement confirms that both the target and foil tone have behavioral effects on the animal; without this, animals could take a single-tone strategy (i.e., learn to lick only for the target tone or withhold licking for the foil tone).

Hit trial ITIs are 4-5 s (to enable full licking of reward), miss trials are not punished and have an ITI of 2-3 s, false alarm trials are punished with an ITI lasting 7-9 s, and correct rejects immediately moved to the next trial with an ITI of 2-3 s. Lick tube removal is automated using a micromanipulator and licks are detected with an infrared beam. Licking is also monitored using an infrared camera or beam to determine whether presentation of sound stimuli elicited a licking movement from the animal. We have confirmed that the animals are still thirsty immediately after training ends, i.e., the animals would lick for ad-libitum access to water. This confirms that any changes in neuronal responses were not due to “satiety” indicating the animal was no longer thirsty.

For pharmacological experiments, animals will be injected bilaterally with either saline vehicle or receptor antagonists (1 μL of 1 mg/mL) at a depth of ~600 μm below the pial surface. The drug will be allowed to diffuse while the animals were headfixed for 10-15 minutes before behavior was initiated.

Two-photon imaging: Imaging will be performed as in our recent studies[45,61]. Mice will be prepared for cranial window implantation by isoflurane anesthesia (2.0% during induction and surgery, 0.75% during physiology). A craniotomy will be performed over the temporal lobe and the auditory cortex exposed (3 mm craniotomy, centered 1.75 mm anterior to the lambda suture on the ridge line). An adeno-associated virus (AAV) vector encoding the calcium indicator GCaMP6s (AAV1-SYN-GCAMP6s, U. Penn Vector Core) will injected for expression in AI layer 2/3 neurons. Above the injection coordinates, a cranial window will be implanted replacing a circular piece of skull by a glass coverslip (diameter of 3 mm, Warner Instruments) secured in place using a mix of dental cement and Krazy glue. A custom-made stainless-steel headpost (Ponoko) will be affixed to the skull using C&B Metabond dental cement (Parkell). A small burr hole will be drilled anterior to bregma and a ground wire placed under the skull above the pial surface. Each animal is allowed to recover for at least 2-3 weeks, and then habituated to head-fixation for ~1 week. For experiments imaging the activity of axonal projections, we will carefully level the head and drill a small burr hole targeting nucleus basalis or locus coeruleus. We will inject 1.5 µl of AAV1-SYN-FLEX-GCAMP6s (U. Penn vector core) into *ChAT-Cre* mice to specifically target cholinergic neurons or into TH-Cre or Dbh-Cre mice to target noradrenergic neurons. We will inject at a rate of 100 nl/min and wait 15 minutes before extracting the injection needle. We will fill the burr hole with bone wax and seal it with dental cement.

For imaging, 2-photon fluorescence of GCaMP6s will be excited at 900 nm using a mode-locked Ti:Sapphire laser (MaiTai, Spectra-Physics, Mountain View, CA) and detected in the green channel (GFP emission). Imaging will be performed on a multiphoton imaging system (Moveable Objective Microscope, Sutter Instruments) equipped with a water immersion objective (20X, NA=0.95, Olympus) and the emission path shielded from external light contamination. Images will be collected using ScanImage (HHMI). To image auditory cortex, the objective is tilted to an angle of 50-60˚. To record time courses of auditory-evoked neuronal activity, awake animals are head-fixed under the microscope and a speaker placed adjacent to the microscope (microphone-ear distance ~10 cm). During auditory stimulation, ~300 μm^2 areas in layer 2/3 of A1 containing multiple GCaMP6s expressing neurons will be selected and imaged (scan rate ~4 Hz, 0.26 s/frames, laser power ≤40 mW).

For motion artifacts, we use a multi-step approach to compensate for x-y motion due to movements of the awake mouse. In brief, we perform a frame-by-frame, rigid registration of the imaging frames that accounts for x-y changes in displacement, rotation and translation. In animals with a structural marker (tdTomato in PV+, SST+, and VIP+ interneurons) we separate the functional channel (green, GCaMP6s) from the structural channel (red, tdTomato). Negative deflections in the fluorescent traces are assumed to be due to motion out of the z-plane. To remain conservative in our data inclusion criteria, any imaging planes that exhibited greater than 5% of cells with statistically-defined negative deflections (pairwise t-test of baseline versus negative peak) will be excluded from the analysis. At all stages of motion correction process, manual inspection is performed to identify motion out-of-the-plane that we are unable to correct by the above approach. In these rare cases, these data will be excluded from further analysis.

For auditory stimulation, we will use pure tones (4-96 kHz at 0.5-1 octave intervals, 100-500 ms duration, 3 ms cosine ramped onsets, 10-80 dB SPL intensity, 0.5-1.0 Hz rate) or FM sweeps (upsweeps from 4-96 kHz or downsweeps from 96-4 kHz, at rates of 1,2,4,8,16,32, and 64 octaves/s, 500 msec in duration), in background white noise at a level of 40 dB SPL. Stimuli will be presented in pseudo-random sequence delivered by a calibrated speaker. These procedures and stimuli are similar to our published studies[28,36,41-43,45,61,62].

In vivo whole-cell recordings: In vivo whole-cell recordings will be performed in awake, head-fixed mice in a sound-attenuating chamber, and recordings obtained from AI neurons with a Multiclamp 700B amplifier (Molecular Devices). For voltage-clamp recordings, pipettes will contain: 130 Cs-methanesulfonate, 1 QX-314, 4 TEA-Cl, 0.5 BAPTA, 4 MgATP, 20 phosphocreatine, 10 HEPES, pH 7.2. Data will be filtered at 5 kHz, digitized at 20 kHz, and analyzed with Clampfit 10 (Molecular Devices). Recordings will be excluded from analysis if Rs or Ri changes >30% relative to baseline. For 2-photon targeted recordings, we will utilize a preparation which enables access to auditory cortex via a ~200 µm hole in a 3 mm piece of round glass, providing the stability required to hold cells across conditions.

For release of endogenous neuromodulators via optogenetic stimulation, blue light will be delivered to the brain surface via a 200 µm fiber (Thorlabs) positioned ~0.5 mm above the surface, coupled to a 473 nm laser (CrystaLaser). During whole-cell recordings, blue light pulses will be triggered 100 ms before stimulus onset and last for 1 s (473 nm wavelength, 2-2.5 mW) at the surface of the brain. Changes in synaptic responses will be compared by Student's paired two-tailed t-tests assuming data passes Kolmogorov-Smirnov normality tests.

Chronic in vivo multi-electrode recordings: Mice will be implanted with microdrive arrays (Versadrive-8 Neuralynx) in either nucleus basalis or locus coeruleus. For surgery, animals will be anesthetized with ketamine (40 mg/kg) and dexmedetomidine (0.125 mg/kg). A craniotomy will be performed, and the location of primary auditory cortex will first be determined by mapping multiunit responses 500-700 µm below the surface using tungsten electrodes in a sound-attenuating chamber. Stainless steel screws and dental cement will be used to secure the microdrive to the skull, and one screw used as ground. Each drive consists of 8 independently adjustable tetrodes. Tetrodes are made by twisting and fusing four polyimide-coated nichrome wires (Sandvik Kanthal; wire diameter 12.5 µm). The tip of each tetrode will be gold-plated to an impedance of 200-500 kOhms at 1 kHz (NanoZ, Neuralynx). After animal recover from surgery (~7 days), recordings will begin after testing baseline retrieval abilities. Animals will be monitored daily by NYU veterinary staff and the member of my lab who performed the surgery, with analgesics and antibiotics provided as necessary.

Tetrodes will be advanced ~60 µm 12 hours prior to each recording session. For recording, signals are first amplified onboard using a small 16-bit unity-gain preamplifier array (CerePlex M, Blackrock Microsystems) before reaching the acquisition system. Spikes are sampled at 30 kS/sec and bandpass filtered between 250 Hz and 5 kHz. Data are digitized and all above-threshold events with signal to noise ratios > 3:1 stored for offline spike sorting. Single units will be identified on each tetrode using OfflineSorter (Plexon Inc.) by manually classifying spikes projected as points in 2D or 3D feature space. The parameters used for sorting include the waveform projection onto the first two principal components, energy, and nonlinear energy. Artifacts are rejected based on refractory period violations (< 1 msec). Clustering quality will be assessed based on the Isolation Distance and Lratio sorting quality metrics. Spontaneous average firing rate will be established by averaging spikes in a 500 msec time window immediately prior to stimulus onset on each trial. To quantify stimulus-evoked responses, we will calculate z-scores of changes in spike count during stimulus presentation: $z = \mu/\sigma$, where μ is the mean change in spike count and σ is the standard deviation of the change in spike count.

Histology: We will perform immunohistochemistry to confirm specific expression of transgenes. Mice will be transcardially perfused using ~45 mL PBS followed by ~ 45 mL 4% PFA under deep anesthesia. Brains are extracted from the skull and post-fixed in 4% PFA for ~2 hours at 4º C, then cryoprotected overnight in 30% sucrose solution and sliced coronally with 16 µm thickness on a cryostat. Fixed brain sections are washed with PBS, then incubated for 2-3 hours at room temperature in blocking buffer containing PBS with 0.5% v/v Triton X-100 (Sigma), 2% v/v normal goat serum (Life Technologies) and 50 mg/mL BSA. The blocking solution will be aspirated and slices incubated in primary antibody (1:1000 dilution of chicken anti-GFP IgY, catalog # ab13970 (Abcam); 1:200 rabbit anti-ChAT IgG, cat. # AB143 (Millipore); 1:1000 dilution of VIP antibody IgG, product. #20077 (Immunostar); 1:1000 dilution of anti-parvalbumin IgG, code #235 (Swant); 1:500 dilution of anti-somatostatin IgG, clone YC7, cat. #MAB354 (Millipore)) overnight at 4ºC. Afterwards, slices are washed and incubated for 1-2 hours at room temperature in secondary antibody (1:1000 dilution, goat anti-chicken IgY Alexa Fluor 488, cat. # A11039; goat anti-rabbit IgG Alexa Fluor 555, cat. # A21430, both from Life Technologies; 1:1000 dilution of goat anti-mouse IgG Alexa Fluor 555, cat. #A21422 (Life Technologies); 1:500 dilution of goat anti-rat IgG, Alexa Fluor 555, cat. #A21434 (Life Technologies)). Finally, slides are washed, incubated at room temperature in DAPI/Hoechst solution (1:1000 in PBS), washed, then coverslipped using Fluoromount (Sigma). Images are acquired with a 20x air objective on a Zeiss LSM 710 Confocal Microscope (Carl Zeiss Inc.) and cell counts were performed in Image J (NIH).

2. Animal Use Justification: The ultimate goal of these experiments is a deep understanding of how the brain responds and changes during auditory learning, due to acoustic input and cholinergic/noradrenergic modulation interacting within auditory cortex. However, our knowledge of these processes is rather limited, and thus research involving animal tissue is unfortunately still necessary. As we are investigating the effects of direct sensory experience and the modulation of local networks of excitatory and inhibitory neurons by subcortical attentional modulators, cell culture systems cannot be used. Computer simulations and mathematical models will be developed in parallel with our experiments, to help inform future directions and limit the overall number of animal studies that are required. Our collaborative studies with other labs- Claudia Clopath, Tim Vogels, Ken Miller, Mark von Rossum, Jason Hunzinger, and others- attest to these efforts. We also aim to develop more powerful methods for studying these important issues in progressively more efficient animal model experiments. We will work to improve the efficiency and effectiveness of experimental methods to still further reduce the numbers of animal experiments that must be conducted.

These experiments will be conducted in mice, which are ideal experimental animals in which to study the circuitry and plasticity of the auditory cortex. The PI is very experienced in conducting behavioral and

electrophysiological studies in rodents. Mice are widely used for studies of neural circuit function, as it is straightforward to express opsins, DREADDs, fluorophores and Ca^{2+} indicators in vivo for optogenetic stimulation or 2-photon imaging, in combination with electrophysiological and behavioral experiments. Importantly, there is also an extensive existing literature and methodological basis for this research. This means that many experimental questions and technical issues have already been solved, minimizing the amount of animals required for our purposes. Mice serve as excellent models for studies of both cortical processing and plasticity. Results from from mouse studies are widely applicable to studies in other mammals such as carnivores, primates, and humans.

We will minimize the number of animals needed for these studies. First, each study has been designed to minimize the number of animals required for statistical significance. A considerable amount of data can be obtained from a single in vivo whole-cell recording, and multiple recordings can be obtained from each animal. Thus multiple studies can be carried out at once. In addition, the PI has a background and training in statistics. This knowledge will be used to design experiments with the goal of reaching statistical significance in the minimal amount of time and using a minimal number of animals. All statistical analyses are performed in MATLAB or GraphPad Prism 6. Datasets will be tested for normality, and appropriate statistical tests applied (e.g., t-test for normally distributed data, Fischer's exact test for categorical observations, Mann Whitney U-test for non-parametric data, Friedman test with Dunn post-hoc for non-parametric data with repeated measurements).

From prior experience and power analysis calculations performed for prior studies[28,36,41-43,45,61,62], we estimate that to obtain statistically reliable results requires approximately 4-8 animals for experiments involving 2-photon imaging (as hundreds of cells can be imaged per animal), 15-50 animals for experiments involving whole-cell recordings, 4-5 animals for multielectrode recording studies in behaving animals, and another 10-15 animals serving as a control group. In total then, we expect to require 295-540 mice. We breed many of our animals in our own facility to save on animal costs and numbers. Numbers reported here are ~10-20% higher than those reported for statistical purposes in the research strategy section, to account for animals lost during surgery or post-surgical monitoring, poor imaging windows, etc. Given that our lab can perform in vivo recordings and imaging from 1-3 animals/day, we believe that the scope of these experiments and this amount of animal usage is entirely feasible within the five year project duration. Subaim 1a contains six major experiments. The first two experiments will use the same 10-20 animals. Experiment three looks specifically at three interneuron subtypes vs excitatory cells, requiring 40-60 mice. Experiment four requires 10-20 mice. Experiment five requires 5-10 mice. Experiment six uses data from previous experiments. Subaim 1b contains four major experiments. The first two experiments will use animals from Subaim 1a, but we expect to also require another 20-40 mice for whole-cell and cell-attached recordings. The third experiment also uses excitatory and inhibitory neuron mice from Subaim 1a, but we expect to also require another 20-40 mice. The fourth experiment (recording in different layers) we expect to require 20-30 mice. Subaim 2a consists of seven behavioral studies each requiring 5-10 mice, except for the first two studies determining receptor sensitivity, which we expect to each require 10-20 mice. Subaim 2b consists of six experiments; five are behavioral and require 5-10 mice each; the recording and imaging experiments of experiment four will require an additional 10-20 mice. Experiments in Aim 3 involve more technically challenging in vivo imaging and recording experiments from neuromodulatory neurons. Axonal imaging experiments will require 20-40 mice, tetrode recordings will require 10-20 mice, fiber photometry studies will require 10-20 mice, whole-cell recordings will require 30-50 mice, and behavioral studies 20-30 mice.

We will use all appropriate parametric and non-parametric statistical tests for assessing statistical significance. In previous experiments we have conducted, we can usually achieve adequate statistical power (i.e., $p < 0.05$) with the number of animals given above. During an experiment, we will continually evaluate the achieved statistical reliability, and if possible, we will reduce the number of animals used in individual experiments if appropriately high reliability (e.g., $p < 0.01$) is obtained.

3. Veterinary Care: Veterinary care of all animals will be provided by vigilant in-house veterinarians and technicians of the Division of Laboratory Animal Resources (DLAR) at [redacted] Medical. Animals will be monitored daily, including weekends, to ensure that they have enough food and water. Bedding will be changed twice per week. Animals will be provided with toys and objects for environmental enrichment. [redacted] Medical DLAR staff will individually examine animals for signs of weight loss and pathological signs, and alert the PI and [redacted] ACUC in these cases.

4. Procedures for Minimizing Discomfort: Animals will be deeply anesthetized with isoflurane or ketamine/ dexmedetomidine before beginning surgery. Animals will be monitored continuously during recording sessions to assess their general well-being, and with a more specific assessment of any possible problems that might

arise from foreign body reaction or infection. All animals are maintained at a surgical level of anesthesia throughout surgical and electrophysiological procedures. Reflexes and vital signs such as respiration and temperature are monitored and logged throughout these procedures at 15-30 minute intervals, and fluids infused to maintain hydration and blood sugar levels. We will routinely administer prophylactic antibiotics in acute electrophysiological studies, and conduct them using clean surgical procedures throughout to minimize the possibility of having a limited time for data acquisition due to later-developing infection. Each member of the lab will first receive training from [redacted] veterinary staff in the responsible use of animals and correct ways of conducting procedures before beginning experiments. Furthermore, the PIs will personally oversee the initial experiments and animal usage of each scientist in my laboratory, to ensure that procedures are correctly and ethically performed.

5. Method of Euthanasia: After in vivo experiments, animals will be anesthetized and euthanized by overdose of pentobarbital anesthetic, followed by intracardiac perfusion of saline and paraformaldehyde for histological study. All of the experimental procedures proposed here are in accordance with an existing protocol approved by the [redacted] Medical IACUC, and these methods are consistent with the recommendations of the American Veterinary Medical Association Guidelines on Euthanasia.

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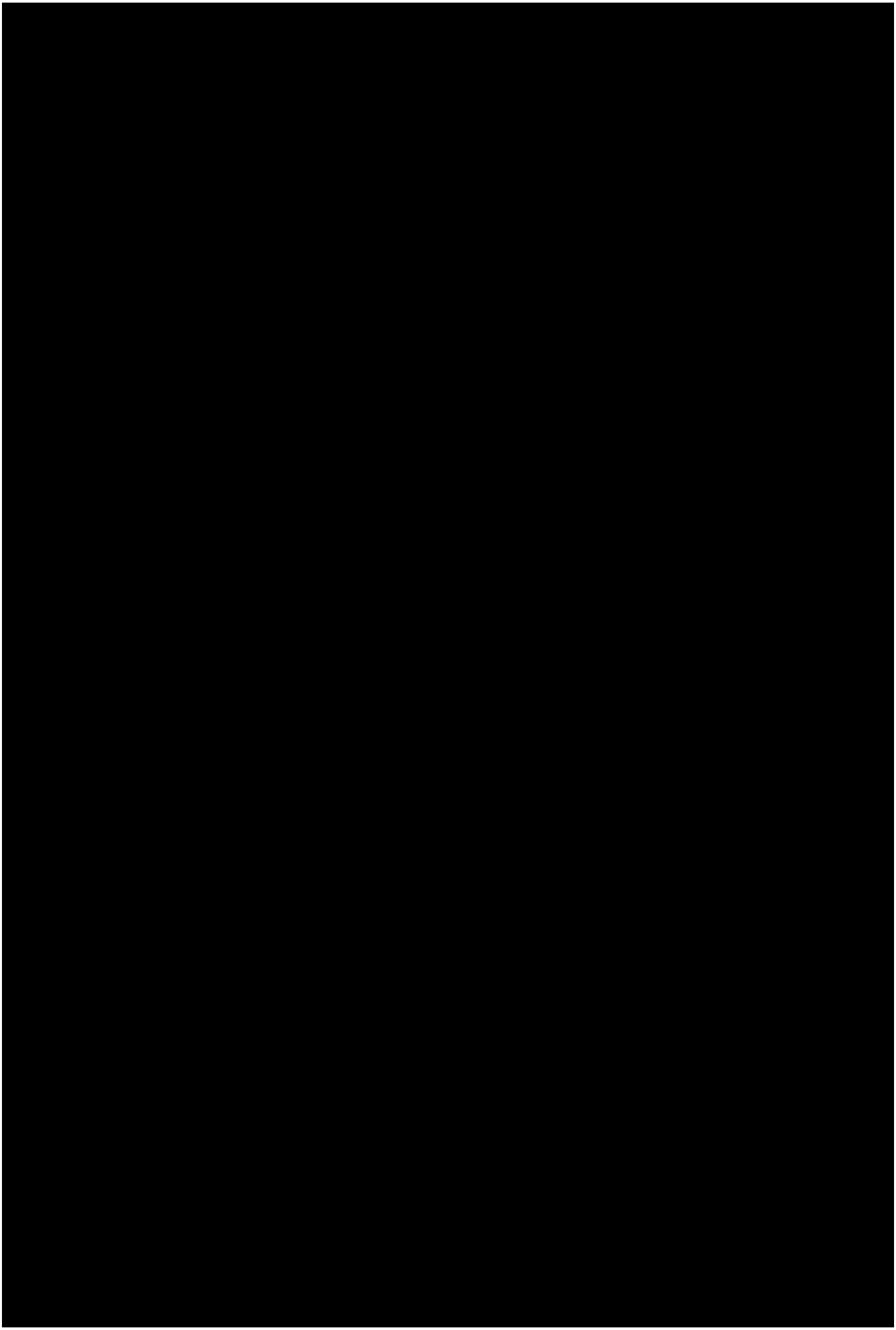
[redacted]



Resource Sharing

We are committed to sharing the data and other resources that are generated in our laboratory. There are three major ways in which we share resources, techniques, and data:

1) We have welcomed several intra- and extramural colleagues to the lab to watch our in vivo whole-cell recording and 2-photon imaging experiments in awake or behaving animals, helping them learn to perform this technique themselves, including students and postdocs from the labs [redacted]

2) The PI has a long history of performing parametric experiments on the requirements for long-term synaptic plasticity, and sharing them with other investigators and laboratories. In particular, we regularly send our results to theoretical neuroscientists for model testing [redacted] We will make our data easily available to other experimental and theoretical investigators, in order to facilitate scientific discourse and ease the speed and reliability of replication. We have an NIH supplement from the National Library of Medicine to work with [redacted] library staff (R01 NIDCD DC12557-S), enabling us to rapidly distribute our published data soon after publication to international collaborators or others who are interested in our results. These published data are now easily available on our lab website, along with Matlab code we have used for simulations in these published studies.

3) We also regularly attend conferences (at least 3/year) to disseminate our new results, and the PI has organized workshops, conference symposia, and frequently gives seminars to extramural institutions (18 invited talks and conference presentations in the 2015-2016 academic year). These include the Society for Neuroscience annual meeting, the International Society for Developmental Psychobiology meeting, Cosyne, and the Association for Research in Otolaryngology midwinter meeting.

Authentication of Key Resources Plan

All resources, chemicals, and reagents used for the experiments in this proposal are standard and supported by numerous publications (e.g., APV to block NMDA receptors, pharmacological reagents, the ChETA variant of channelrhodopsin-2, tdTomato interneuron transgenic mouse lines).