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

APPLICATION FOR FEDERAL ASSISTANCE
**SF 424 (R&R)**

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

**1. TYPE OF SUBMISSION***

❍ Pre-application ● Application ❍ Changed/Corrected Application

**4.a. Federal Identifier**

**b. Agency Routing Number**

**c. Previous Grants.gov Tracking Number**

**2. DATE SUBMITTED** | **Application Identifier**

**5. APPLICANT INFORMATION** **Organizational DUNS*:** 6218898150000

Legal Name*: The Trustees of Columbia University in the City of New York
Department: Neuroscience
Division: Neurobiology and Behavior
Street1*: 3227 Broadway
Street2: Jerome L. Greene Science Center
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 10027-7922

Person to be contacted on matters involving this application
Prefix: First Name*: William Middle Name: Last Name*: Berger Suffix:
Position/Title: Executive Director of Sponsored Projects Admi
Street1*: 630 West 168th Street, Box 49
Street2:
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100323702
Phone Number*: 212-305-4191 Fax Number: Email: grants-office@columbia.edu

**6. EMPLOYER IDENTIFICATION NUMBER** *(EIN) or (TIN)** 135598093

**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***
Behavioral flexibility and the geometry of neural representations in hippocampus and prefrontal cortex

**12. PROPOSED PROJECT**

| Start Date* | Ending Date* |
|---|---|
| 07/01/2021 | 06/30/2024 |

**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
NY-013

# SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE



**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: First Name*: Max Middle Name: Jacob Last Name*: Pensack Suffix:
Position/Title: Graduate Student
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Neurobiology and Behavior
Division: Zuckerman Institute
Street1*: 3227 Broadway, L6, Quad 6A
Street2: Jerome L. Greene Science Center
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100277922
Phone Number*: 212-853-1733 Fax Number: Email*: mjp2143@columbia.edu

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested* | $244,635.00 |
| b. Total Non-Federal Funds* | $0.00 |
| c. Total Federal & Non-Federal Funds* | $244,635.00 |
| d. Estimated Program Income* | $0.00 |

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

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

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

**18. SFLLL or OTHER EXPLANATORY DOCUMENTATION** File Name:

**19. AUTHORIZED REPRESENTATIVE**

Prefix: First Name*: Madhavi Middle Name: Last Name*: Nambiar Suffix: Ph.D
Position/Title*: Senior Project Officer
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Sponsored Projects Administrat
Division:
Street1*: 630 West 168th Street, Box 49
Street2:
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100323702
Phone Number*: 2123054191 Fax Number: Email*: grants-office@columbia.edu

**Signature of Authorized Representative***
Madhavi Nambiar

**Date Signed***
12/08/2020

**20. PRE-APPLICATION** File Name:

**21. COVER LETTER ATTACHMENT** File Name:MJP_F31_Cover_Letter.pdf

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

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

# Project/Performance Site Location(s)

**Project/Performance Site Primary Location**

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

| | |
|---|---|
| Organization Name: | The Trustees of Columbia University in the City of New York |
| Duns Number: | 6218898150000 |
| Street1*: | 3227 Broadway |
| Street2: | Jerome L. Greene Science Center |
| City*: | New York |
| County: | |
| State*: | NY: New York |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 100277922 |
| Project/Performance Site Congressional District*: | NY-013 |

**Additional Location(s)** File Name:





Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T16:53:51.000-05:00

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

# RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A3007-01

**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*** | MJP_F31_Project_Summary.pdf |
| **8. Project Narrative*** | MJP_F31_Project_Narrative.pdf |
| **9. Bibliography & References Cited** | Bibliography.pdf |
| **10.Facilities & Other Resources** | MJP_F31_Facilities_Resources.pdf |
| **11.Equipment** | MJP_F31_Equipment.pdf |





Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T16:53:51.000-05:00

## PROJECT SUMMARY/ ABSTRACT

We live in a world that is constantly changing. In order to pursue goals and survive in such a complex environment, one cannot rely on learned stimulus-response associations alone. Rather, we require behavioral flexibility – the ability to rapidly adjust actions and expectations in response to changes in internal and environmental variables. Our ability to engage in flexible behavior breaks down under conditions of stress and in the setting of many neuropsychiatric conditions, including addiction, anxiety, mood, and psychotic disorders. This project aims to characterize the underlying neurophysiology that gives rise to two important types of behavioral flexibility: Type 1, rapid context-dependent adjustments in behavior, and Type 2, rapid decision-making in novel conditions through generalization from past experiences. These types of flexibility make distinct demands on neural representations. The dorsolateral prefrontal cortex (DLPFC) and hippocampus (HPC) are thought to contribute to both types of flexibility, but delineating each area's unique computational role has proved difficult. The overarching hypothesis of this grant is that neural representations of variables – considered as the pattern of activity in a population across experimental conditions - must exhibit a particular geometry to support the two types of behavioral flexibility. I will test this hypothesis by comparing the geometry of representations during correct and incorrect behavior on certain trial types. By elucidating the neural basis of flexibility in HPC and DLPFC, this project provides a foundation for understanding the neural basis of deficits seen in stress and neuropsychiatric illness, paving the way for the development of future treatments.

## PROJECT NARRATIVE

Pursuing goals in a complex environment requires behavioral flexibility – the ability to rapidly adjust actions and expectations in response to changes in internal and environmental variables. Deficits in flexible behavior are seen in nearly all psychiatric conditions, including addiction, anxiety, mood, and psychotic disorders. The experiments outlined in this proposal will examine how the geometry of neural representations in the hippocampus and dorsolateral prefrontal cortex correlates with behavioral flexibility in healthy monkeys, laying the groundwork for the development of future psychiatric treatments.

## BIBLIOGRAPHY


[1] Rudy JW, O'Reilly RC. Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. *Behav Neurosci* 19991201; 113: 867.

[2] Fusi S, Miller EK, Rigotti M. Why neurons mix: high dimensionality for higher cognition. *Curr Opin Neurobiol* 2016; 37: 66–74.

[3] Rigotti M, Barak O, Warden MR, et al. The importance of mixed selectivity in complex cognitive tasks. *Nature* 2013; 497: 585--590.

[4] Badre D, Bhandari A, Keglovits H, et al. The dimensionality of neural representations for control. *Curr Opin Behav Sci* 2021; 38: 20–28.

[5] Wallis JD, Anderson KC, Miller EK. Single neurons in prefrontal cortex encode abstract rules. *Nature* 2001; 411: 953--956.

[6] Saez A, Rigotti M, Ostojic S, et al. Abstract context representations in primate amygdala and prefrontal cortex. *Neuron* 2015; 87: 869--881.

[7] Antzoulatos EG, Miller EK. Differences between neural activity in prefrontal cortex and striatum during learning of novel abstract categories. *Neuron* 2011; 71: 243--249.

[8] Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* 2001; 24: 167–202.

[9] Mante V, Sussillo D, Shenoy KV, et al. Context-dependent computation by recurrent dynamics in prefrontal cortex. *Nature* 2013; 503: 78–84.

[10] Constantinescu AO, O'Reilly JX, Behrens TE. Organizing conceptual knowledge in humans with a gridlike code. *Science* 2016; 352: 1464--1468.

[11] Sakon JJ, Suzuki WA. A neural signature of pattern separation in the monkey hippocampus. *Proc Natl Acad Sci* 2019; 116: 9634--9643.

[12] Whittington JCR, Muller TH, Mark S, et al. The Tolman-Eichenbaum Machine: Unifying Space and Relational Memory through Generalization in the Hippocampal Formation. *Cell* 2020; 183: 1249-1263.e23.

[13] Gulli RA, Duong LR, Corrigan BW, et al. Context-dependent representations of objects and space in the primate hippocampus during virtual navigation. *Nat Neurosci* 2020; 23: 103–112.

[14] Rolls E. The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci*; 7. Epub ahead of print 2013. DOI: 10.3389/fnsys.2013.00074.

[15] Bernardi S, Benna MK, Rigotti M, et al. The Geometry of Abstraction in the Hippocampus and Prefrontal Cortex. *Cell* 2020; 183: 954-967.e21.

[16] Kriegeskorte N, Kievit RA. Representational geometry: integrating cognition, computation, and the brain. *Trends Cogn Sci* 2013; 17: 401–412.

[17] Arnsten AFT. Stress weakens prefrontal networks: molecular insults to higher cognition. *Nat Neurosci* 2015; 18: 1376–1385.

[18] Benzina N, Mallet L, Burguière E, et al. Cognitive Dysfunction in Obsessive-Compulsive Disorder. *Curr Psychiatry Rep* 2016; 18: 80.

[19] Koob GF, Volkow ND. Neurocircuitry of Addiction. *Neuropsychopharmacology* 2010; 35: 217–238.

[20] Waltz JA. The neural underpinnings of cognitive flexibility and their disruption in psychotic illness. *Neuroscience* 2017; 345: 203–217.

[21] Park J, Moghaddam B. Impact of anxiety on prefrontal cortex encoding of cognitive flexibility. *Neuroscience* 2017; 345: 193–202.

[22] Behrens TEJ, Muller TH, Whittington JCR, et al. What Is a Cognitive Map? Organizing Knowledge for Flexible Behavior. *Neuron* 2018; 100: 490–509.

[23] DiCarlo JJ, Zoccolan D, Rust NC. How Does the Brain Solve Visual Object Recognition? *Neuron* 2012; 73: 415–434.

[24] Wallis JD, Anderson KC, Miller EK. Single neurons in prefrontal cortex encode abstract rules. *Nature* 2001; 411: 953–956.

[25] Miller EK, Nieder A, Freedman DJ, et al. Neural correlates of categories and concepts. *Curr Opin Neurobiol* 2003; 13: 198–203.

[26] Saez A, Rigotti M, Ostojic S, et al. Abstract Context Representations in Primate Amygdala and Prefrontal Cortex. *Neuron* 2015; 87: 869–881.

[27] Fusi S, Miller EK, Rigotti M. Why neurons mix: high dimensionality for higher cognition. *Curr Opin Neurobiol* 2016; 37: 66–74.

[28] Barak O, Rigotti M, Fusi S. The Sparseness of Mixed Selectivity Neurons Controls the Generalization–Discrimination Trade-Off. *J Neurosci* 2013; 33: 3844–3856.

[29] Johnston WJ, Palmer SE, Freedman DJ. Nonlinear mixed selectivity supports reliable neural computation. *PLOS Comput Biol* 2020; 16: e1007544.

[30] Chung JE, Magland JF, Barnett AH, et al. A Fully Automated Approach to Spike Sorting. *Neuron* 2017; 95: 1381-1394.e6.

[31] Fusi S, Asaad WF, Miller EK, et al. A Neural Circuit Model of Flexible Sensorimotor Mapping: Learning and Forgetting on Multiple Timescales. *Neuron* 2007; 54: 319–333.

[32] Milner B, Squire LR, Kandel ER. Cognitive Neuroscience and the Study of Memory. *Neuron* 1998; 20: 445–468.

[33] Eichenbaum H. Hippocampus: Cognitive Processes and Neural Representations that Underlie Declarative Memory. *Neuron* 2004; 44: 109–120.

[34] Wirth S, Yanike M, Frank LM, et al. Single Neurons in the Monkey Hippocampus and Learning of New Associations. *Science* 2003; 300: 1578–1581.

[35] Schapiro AC, Turk-Browne NB, Norman KA, et al. Statistical learning of temporal community structure in the hippocampus. *Hippocampus* 2016; 26: 3–8.

[36] Kumaran D, Summerfield JJ, Hassabis D, et al. Tracking the Emergence of Conceptual Knowledge during Human Decision Making. *Neuron* 2009; 63: 889–901.

[37] Buckley MJ, Mansouri FA, Hoda H, et al. Dissociable Components of Rule-Guided Behavior Depend on Distinct Medial and Prefrontal Regions. *Science* 2009; 325: 52–58.

[38] Antzoulatos EG, Miller EK. Differences between Neural Activity in Prefrontal Cortex and Striatum during Learning of Novel Abstract Categories. *Neuron* 2011; 71: 243–249.

[39] Wutz A, Loonis R, Roy JE, et al. Different Levels of Category Abstraction by Different Dynamics in Different Prefrontal Areas. *Neuron* 2018; 97: 716-726.e8.

[40] Brown MW, Aggleton JP. Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* 2001; 2: 51–61.

[41] Jutras MJ, Buffalo EA. Recognition memory signals in the macaque hippocampus. *Proc Natl Acad Sci* 2010; 107: 401–406.

[42] Ninokura Y, Mushiake H, Tanji J. Integration of Temporal Order and Object Information in the Monkey Lateral Prefrontal Cortex. *J Neurophysiol* 2004; 91: 555–560.

## FACILITIES & OTHER RESOURCES

***Zuckerman Mind Brain Behavior Institute (ZMBBI), in the Jerome L. Greene Science Center:*** The Salzman lab belongs to the Zuckerman Mind Brain Behavior Institute (ZMBBI) in the new Jerome L. Greene Science Center, a state-of-the-art facility designed by celebrated architect Renzo Piano at 125th and Broadway in Manhattanville. The 9-story, 450,000 square-foot structure is designed to maximize creative collaboration among scientists. It includes connecting stairways and common spaces that link individual researchers and lab groups into a coherent community. Bringing together researchers from Columbia University Medical Center, the Faculty of Arts and Sciences, the Fu Foundation School of Engineering and Applied Science, and other schools on the Morningside Campus, the Greene Science Center has become a hub of cross-university research that it was envisioned to be. The new science center brings 56 interdisciplinary groups together under one roof, along with outstanding facilities and technical support to tackle major questions related to brain and behavior. Located adjacent to the Greene Center is the University Forum and Academic Conference Center, which features a state-of-the-art 430-seat auditorium situated above a 2,000-square-foot lobby on the ground floor. The Forum hosts academic conferences, meetings and symposia, providing a space where leaders in neuroscience and other fields can come together to share ideas.

Concerning systems and theoretical neuroscience in particular, the intellectual environment at Columbia University is world-class. Faculty members with related interests include Michael Shadlen, Michael Goldberg, Jacqueline Gottlieb, Vince Ferrera, Mark Churchland, Aniruddha Das, Daphna Shohamy, Nikolaus Kriegeskorte, Richard Axel, Rui Costa, Stefano Fusi, Liam Paninski, John Cunningham, Larry Abbott, and Kenneth Miller. The Neurobiology and Behavior Program at Columbia is internationally respected and attracts excellent graduate students, and the institution houses many talented postdoctoral fellows.

***Salzman Lab*:** Dr. Salzman's laboratory is housed primarily in Quad 6A of the Greene building (total 10,734 sq. ft.), consisting of open labs currently shared with Drs. Mark Churchland and Yasmine El-Shamayleh. The lab is also located near the laboratories of Drs. Shadlen, Goldberg, Ferrera, Gottlieb and Das, all of which utilize awake, behaving monkeys for experimentation. The Salzman lab also occupies the same floor as the Center for Theoretical Neuroscience, and interactions between members of the Salzman lab and those of the Center, (especially Dr. Fusi's group) are common. Dr. Salzman's dedicated lab space in Quad 6A is estimated at 2,662 sq. ft. including office space. The space contains support areas for all experimental work: animal prep areas, electrode manufacturing stations, electronics work benches, a small mechanical workbench, a bench for histological processing, as well as some computer workstations. In addition, Dr. Salzman maintains space on quad 7B (approximately 276 square feet), which contains additional bench space for molecular and histological work in rodents. Finally, Dr. Salzman's lab makes use of the core facility space described below.

A break space and large conference room are available to all four quads on each floor, and are 1763 sq. ft. Within the building, there are numerous 'interaction' spaces in the corners of each quad where individuals are free to come together and share ideas. Additionally, there are many conference rooms - at least one small, medium, and large per paired floor - that can be reserved by utilizing the online booking system.

***Offices*:** Dr. Salzman has dedicated office space (totaling 310 sq. ft.) for his office and administrative staff. Desk space for students, fellows, and other scientific staff are included in the space described for the lab.

***Computers:*** See equipment.

***Animals*:** In addition to the laboratory space described above, there is animal housing space on quad 6A in a physically separated, secure animal facility. This space is AAALAC approved and is located adjacent to a cage washing facility. Sterile surgery is conducted in a designated animal surgical facility administered by the animal facility on floor SC4 of the Greene building. Veterinarians and animal health technicians are available on a 24-hour basis to address any clinical problems that may arise with the experimental animals. Dr. Christina Winnicker is the Associate Director and lead veterinarian on-site. All procedures and oversight relating to animal work will be under the aegis of Columbia University's IACUC and Institute for Comparative Medicine.

The animal care and use program at Columbia University (CU) is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and has an assurance with

the Public Health Service (PHS), assurance number A3007-01. The full-time dedicated veterinary staff has training and experience with the species used in the CU research programs and is available 24 hours a day, 7 days a week. Animals are checked daily at a minimum, but more frequently as needed. An intensive care unit is available, which can provide continuous monitoring and medical care, if needed. Complete surgical and imaging facilities are also available. Within the CU program, the Attending Veterinarian or his delegates are authorized, by the Institutional Official, to intervene to alleviate pain or distress as deemed appropriate.

The *in vivo* primate work on this project will occur in the dedicated electrophysiology rooms which are adjacent to the animal housing space on quad 6A.

***Fusi Lab:*** The laboratory conducts theoretical work through Columbia's Center for Theoretical Neuroscience, housed in a 10,200 square foot area within the Greene Science Center. The theory space in this building consists of two floors connected by an inner staircase, an arrangement that increases the number of our experimental laboratory neighbors. We have access to numerous meeting spaces, conference rooms of various sizes both within our space and immediately outside it, and a larger auditorium.

***Offices:*** All of the theory investigators have their own offices in the new building. Graduate students and postdocs choose between large, open shared office spaces or smaller shared, but more private, offices.

***Computers:*** All theory researches have their own GPU machines and laptops. In addition, all have access to ZMBBI and Columbia high-performance computing clusters. ZMBBI has purchased "Habanero", a new high performance computing cluster consisting of 222 nodes. Although we will have shared access to all of the cluster, 5 nodes with 128 GB memory and 12 cores each, and 2 nodes with 512GB memory and 12 cores each are reserved specifically for Theory Center researchers at all levels. We also have free access to Columbia's High Performance Computing Facility, in particular the “Yeti” cluster with over 2600 CPU and about 20K GPU cores and 160 TB working storage. This facility will expand continually in the coming years. Additional major computational resources (~6500 CPU cores, ~1.5 PB storage) are available for rent at reduced cost for Columbia researchers (“ARCS”).

***ZMBBI Core Facilities:***

The ***Research Computing Platform*** (Rajendra Bose, PhD, Director) is a five-person team that provides leading edge research computing services and infrastructure to all members of the Zuckerman Institute as well as the other Platforms within the Jerome L. Greene Science Center.

The Institute possesses a dedicated, shared, multi-petabyte storage system (“Engram”) to house images and other research data, and allow collaboration by Zuckerman researchers. This enterprise-grade storage platform has a current capacity of two usable petabytes (PB, equal to 2000 terabytes (TB)) and is capable of accommodating tens of PB of growth. Three different levels of storage include remote backup for disaster recovery purposes.

The Research Computing group manages a specialized computing cluster (“Axon”) with some of the latest graphics processing unit (GPU) processors to enable machine learning and other specific neuroscience applications for Zuckerman labs at a greater scale than can be achieved on individual workstations, some of which cannot be feasibly done on more traditional central processing unit (CPU)-based infrastructure.

In addition, the group also provides Zuckerman researchers with access to the university’s general shared high-performance computing (HPC) clusters, launched in November 2016 and December 2018. These clusters respectively have over 300 and 150 servers with high speed interconnect and parallel file systems. Research Computing also offers an on-demand computing service (“Cortex”) that provides easy-to-use private servers for labs and researchers that can directly connect to Engram data storage.

Engram, Axon, Cortex and other infrastructure are housed in the dedicated, climate-controlled 1400 square foot Zuckerman Institute Data Center in the Jerome L. Greene Science Center with the ability to power and cool 30 cabinets of equipment. The computational resources in the Zuckerman data center are accessed through a data network that has up to 10 Gigabit per second (Gbps) data transfer speeds within the building, and that connects

to the 40 Gbps campus backbone network and beyond. Columbia maintains a 40 Gbps regional and national (NYSERNet and Internet2) connection with access to international research networks, as well as multiple 20 Gbps connections to the public Internet.

The ***Advanced Instrumentation Platform*** (Tanya Tabachnik, Director) designs, builds and tests instruments, tools, and software that are not commercially available. The group has complementary skills spanning mechanical, electrical, and optical engineering including manufacturing. The Advanced Instrumentation platform supports an in-house professional machine and fabrication shop, as well as a small shop that can be used directly by the researchers themselves, after completing a short training course. The Platform is continuously improving its engineering and manufacturing capabilities to allow rapid and cost-effective delivery of functional solutions.

In all cases, the platform's philosophy is to collaborate closely with the research community to understand the neuroengineering problem to be solved. We believe that the synergy between life science and engineering will bring next-generation design solutions. We support shared use of equipment and software by all interested researchers. The Platform's goal is to provide in-house solutions and training to assist researchers through a complete life cycle of system development.

The Advanced Instrumentation platform is located with the Jerome L Greene Science Center encompassing 2,200 Sq. feet in the lower level and also has additional space at the laboratory 3 level. Facilities include a fully equipped modern machine shop as well as electronic shop and engineering area. The shop includes a storage space, and two manufacturing and assembly spaces. The Manufacturing Shop is equipped with a state-of-the-art 3-axis CNC Mill as well as manual milling machine, manual lathe, table and vertical saws, drill press, laser cutters and several 3D printers. Electrical equipment includes a Digital Storage Oscilloscope 2Gs/s, with 4 channels capable of 100MHz and FFT Mathematical analysis, Logic Analyzer of 16 Channels at 100MHz, decoding of I2C, SPI, RS232 interfaces, 25MHz Math Function and Arbitrary Signal Generator, Triple Channel intelligent programmable DC Power Supply 30V 1.5A, 5V 6A, with max load of 120W, 300 ft. Electrical and communications TDR Hand held meter, hot Air rework soldering station. The Facility also has a metrology and optical equipment, shared with the Cellular Imaging Platform, suitable for optical design, integration, and assembly.

***Magnetic Resonance Imaging Center*** (John T. Vaughan, PhD, director): The Zuckerman Mind, Brain, Behavior Institute in the new Jerome L. Greene Science Center is home to three new, state-of-the-art, research-grade MR systems and the laboratories and staff to support their application to preclinical and human biomedical investigations. All systems are fully capable of MRI, multi-nuclear MRS and fMRI. All systems will be fully dedicated to research.

MR Systems: Two Siemens, Prisma 3T clinical bore systems and a Bruker Biospin 9.4T preclinical system have recently been installed on the basement floor of the Greene building. We are also anticipating purchase or a 7T human system (a request for proposals has been tendered with a 12-month delivery date anticipated).

***NeuroNex Theory Team and the Columbia Center for Theoretical Neuroscience:*** The newly inaugurated NSF NeuroNex Theory Team Hub provides theoretical support and guidance through all stages of discovery in neuroscience: Formulating goals and directions for experimental design; Extracting signals from noisy data, including electrophysiology, imaging, genetics and connectomics; Developing and applying methods for isolating the most informative signals from high-dimensional data; Constructing models, ranging from abstract to detailed, to identify the neural mechanisms underlying phenomena being studied; and Extracting lessons and principles that codify what has been learned and provide a framework for returning to the first item on this list and iteratively advancing the field.

Led by PI Laurence Abbott, with co-PIs: Liam Paninski, John Cunningham, Kenneth Miller, and Stefano Fusi, the team is engaged in collaborations on projects in the following areas: Methods for acquiring signals from electrophysiology and calcium imaging; Analysis and dimensional reduction of neural data; Inference of network structure from activity and connectome data; Modeling how information migrates from one area to another, including the transfer of memories from hippocampus to prefrontal cortex and the transfer of task expertise from prefrontal cortex to striatum; Comparative studies of primary visual cortex of rodents and primates and olfactory

processing of flies and mice; and Modelling various forms of memory, ranging from working to episodic and long-term, using data from human subjects.

A major goal of the neuroNex Theory hub is to provide the links needed to extract insights that might get lost without strong theoretical support due to fragmentation between different laboratories. We stress areas where theoretical work can enhance experimental output and link together studies done in different brain areas and on different species.

## EQUIPMENT

Dr. Salzman's lab contains all the necessary equipment to conduct neurophysiological experiments. Custom assembled desktop PCs running Windows are at the core of each existing experimental apparatus. Data acquisition systems run a software package called MonkeyLogic, developed by the NIH for visual stimulus presentation, behavior control, stimulus control, data collection, and data storage. Online data analysis in MATLAB, as well as user interface windows, can occur. Visual stimuli are presented on video monitors. Single unit data are recorded using either NAN multielectrode drives or a Narashige hydraulic microdrive, in conjunction with Plexon multichannel data acquisition systems for amplifying, filtering, and sorting spikes and for saving LFPs and all other data. The lab has 2 16-channel Plexon systems, 1 64-channel Plexon system, and 2 32-channel Blackrock systems. In addition, the Salzman lab has recently acquired a 128 channel Bio-Signal system for recording and stimulation, which will be available for this project. Neuronal signals are visually displayed on Tektronix oscilloscopes and are additionally fed into an audio monitor. Monkeys' eye movements are monitored using an infrared eye monitoring system by SR Systems (EyeLink). Each rig also contains various custom-made electronic devices such as computer interfaces, amplifiers, filters, and differentiators. In addition, the Salzman lab uses a BrainSight system for MRI-guided surgical procedures. This system provides on-line feedback during surgical procedures so that chamber placement, etc., can be visualized on registered MRIs in the operating room. Shared resources at Columbia provide a surgical microscope (Zeiss) and other smaller microscopes for examining histological sections and preparing electrodes.

The Salzman lab also maintains an off-line computer facility with Windows, Apple, or Linux computers for data analysis, software development, and computational modeling, as well as laptop computers for scientific presentations. Data analysis is performed on these computers, typically using customized MATLAB software for statistical analysis of data. Cluster computing services are also available through Columbia University for more computationally demanding tasks, such as offline sorting of multiple channels with automatic algorithms. In addition, the computers are used for word processing, graphical data display, and PowerPoint presentation preparation. The Salzman lab also maintains a brand-new 120 TB Synology Data Storage system, which can be accessed from each peripheral workstation to ensure data security and backup.

The equipment above is available to this project at no direct cost to the Government.

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

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Max Middle Name Jacob Last Name*: Pensack Suffix:

Position/Title*: Graduate Student
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Neurobiology and Behavior
Division: Zuckerman Institute
Street1*: 3227 Broadway, L6, Quad 6A
Street2: Jerome L. Greene Science Center
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 100277922

Phone Number*: 212-853-1733 Fax Number:

E-Mail*: mjp2143@columbia.edu

Credential, e.g., agency login: MAX.JP

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

Degree Type: MD,PHD,BA Degree Year: 2025,2024,2011

Attach Biographical Sketch*: File Name: MJP_F31_Biosketch.pdf

Attach Current & Pending Support: File Name:



Tracking Number: GRANT13256070 Funding Opportunity Number: PA-21-051 . Received Date: 2020-12-08T16:53:51.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: C. Middle Name DANIEL Last Name*: SALZMAN Suffix:

Position/Title*: Professor
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Neuroscience
Division: Zuckerman Institute
Street1*: 3227 Broadway, L6, 6A
Street2: Jerome L. Greene Science Center
City*: NEW YORK
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 100277922

Phone Number*: 212-853-1186 Fax Number: 212-543-5816

E-Mail*: CDS2005@COLUMBIA.EDU

Credential, e.g., agency login: SALZMAND

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

Degree Type: MD,PHD,BA Degree Year: 1995,1995,1985

Attach Biographical Sketch*: File Name: Biosketch_Salzman_training_MAX.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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

Position/Title*: Professor
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Neuroscience
Division: Zuckerman Institute
Street1*: 3227 Broadway, L6, 6D
Street2: Jerome L. Greene Science Center
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 10027-7922

Phone Number*: 212-853-1076 Fax Number:

E-Mail*: sf2237@columbia.edu

Credential, e.g., agency login: SF2237

Project Role*: Other (Specify) Other Project Role Category: Co-Sponsor

Degree Type: PHD Degree Year: 1999

Attach Biographical Sketch*: File Name: MJP_F31_Biosketch_Fusi.pdf

Attach Current & Pending Support: File Name:

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

# BIOGRAPHICAL SKETCH

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

NAME: Pensack, Max Jacob

eRA COMMONS USER NAME (credential, e.g., agency login): MAX.JP

POSITION TITLE: Graduate Research Assistant

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 & LOCATION | DEGREE | END DATE | FIELD OF STUDY |
|---|---|---|---|
| Columbia University, New York, NY | BA | 05/2011 | Philosophy |
| Columbia University, New York, NY | OTH | 05/2016 | Postbac. Cert. in Premedical Sciences |
| Columbia University, New York, NY | PHD | *Present* | Neurobiology and Behavior |
| Columbia University, New York, NY | MD | *Present* | Medicine |

## A. Personal Statement

I am pursuing doctoral training in neurobiology and behavior in order to prepare for my career as a physician-scientist, uncovering mechanisms that give rise to our mental life. Thought, feeling, and behavior are extremely complex and interrelated phenomena, and characterizing their physiological basis in the brain is an important goal - both in developing better treatments for neuropsychiatric disease and in understanding the human condition from a scientific point of view. My current trajectory and non-traditional background make me uniquely qualified to work on these complex problems in my career as a principal investigator.

I came to neuroscience and medicine through my earlier interests in the arts and humanities. As an undergraduate at Columbia University, I majored in philosophy and was heavily involved in college theater as an actor and director. I loved philosophy for all its arguments, ideals, and emphasis on rigor, and I loved theater for its ability to explore society, freedom, and the emotions. In my senior year, I directed the school's fall musical, which involved coordinating over 50 people between the cast, crew, musicians, and creative team. Through such projects, I experienced the joys of working with a small team to tell a shared story. I have since experienced similar joys in my research career, telling data-driven stories.

After college I wanted to experience life abroad, so I applied to work for the Japan Exchange and Teaching Program – an inter-governmental initiative that employs foreign graduates as English teachers in Japanese public schools. For two years, I lived and worked in a rural town of 10,000 people in southwestern Japan. I took great pride and pleasure in working in the public sector, and I found teaching to be highly rewarding. Also, during this time I started reading in the philosophy of mind literature. Philosophy of mind is perhaps the most important field in philosophy today, as its findings have strong implications for the field at large. However, these theories are all highly dependent on current scientific understanding. I realized that if I wanted to understand the mind, I would need to start studying the brain.

To this end, I started pursuing a career in medicine, hoping my studies could be applied directly in the service of alleviating human suffering. I enrolled in Columbia's Postbaccalaureate Premed Program and sought out my first research experiences in neuroscience. I became involved with two Alzheimer's disease projects at Weill Cornell Medical College - one performing slice electrophysiology experiments on the hypothalamus in Dr. Constantino Iadecola's lab, and one conducting clinical neurology research with Dr. Alon Seifan on the connection between developmental learning disabilities and atypical forms of dementia. These projects introduced me to many technical and conceptual tools, but I quickly learned that I would also need to develop computational skills if I hoped to do advanced work in the field. To do so, I secured a position in January of 2016 working with Prof. Lawrence Sirovich, an applied mathematician at The Rockefeller University. The learning curve was steep, but I quickly became engrossed by quantitative work. Our project focused on disease prediction using genome-wide association study (GWAS) data, and I saw for the first time how quantitative models could potentially impact the lives of many people in a positive way.

By the time I started medical school in September of 2016, I knew I wanted research to be a major part of my career. During that year I rotated in several labs, and secured a summer fellowship with Dr. Tadashi Isa at Kyoto University, where I studied the neural circuitry underlying blindsight in macaque monkeys. During my stay, I saw for the first time how non-human primates offer unique access to the study of complex behaviors and mental processes. Evolution in these animals, just as in humans, gave rise to remarkable faculties that regulate

attention, decision-making, and emotions. Primate work seemed like the perfect arena for me to combine my prior interests with my new skills in computation, research, and clinical medicine.

When I returned to Columbia, I immediately sought out primate labs and explored options for doctoral-level training. I was fortunate to meet Dr. Daniel Salzman, who has been very supportive of me and my new direction. He recommended that I apply for Columbia's PhD program in neurobiology and follow the MD/PhD curriculum piecewise. Ironically, Dr. Salzman himself was a philosophy major, a student in Columbia's postbac program, and a traditional MD student before pursuing doctoral training in Bill Newsome's lab. Hearing Dr. Salzman's trajectory gave me confidence that I would find a rewarding path in neuroscience and medicine.

Since joining the Salzman lab, I have continued to integrate my former interests through research on the limbic system and interconnected areas of the prefrontal cortex. Our work studies the bidirectional interactions between the cognitive and evaluative systems of the brain. My project expands a line of research recently developed through collaborations with Dr. Stefano Fusi's theoretical group, concerning the geometric structure of neural representations and behavioral flexibility. Although this work focuses on the physiology of healthy brains, I know our findings will have implications for neuropsychiatric conditions as well.

Successful scientists need to focus on important questions and convey their findings through clear verbal and written communication. My background in philosophy and the performing arts has equipped me with strong analytic and communication skills and has motivated many of my questions about mind, brain, and behavior. My training in medicine has only deepened these interests, while keeping service to humanity always in perspective. Through my training in the Salzman lab and as a member of the neuroscience community at Columbia, I will continue to develop a sense of rigor and a nose for important scientific questions, using my career to contribute what I can to our emerging understanding of the human psyche in health and disease.

## B. Positions and Honors

### Positions and Employment

| | |
|---|---|
| 2009 - 2009 | Undergraduate Research Asst., Columbia U. – Dept. of Psychology, PI: Donald Hood |
| 2012 - 2014 | English Teacher, Japan Exchange and Teaching (JET) Program, Kumamoto Prefecture |
| 2014 - 2015 | Research Asst., Weill Cornell Medical Col., Memory Disorders Program, PI: Alon Seifan |
| 2014 - 2015 | Research Asst., Weill Cornell Medical Col., Brain & Mind Research Institute, PI: Gang Wang |
| 2016 - 2016 | Research Asst., The Rockefeller U., Knight Biophysics Laboratory, PI: Lawrence Sirovich |
| 2017 - 2017 | Research Asst., Kyoto University, Dept. of Neuroscience, PI: Tadashi Isa |
| 2018 - | Graduate Student, Columbia U., Program in Neurobiology & Behavior, PI: Daniel Salzman |
| 2019 - | Teaching Asst., Columbia University, Dept. of Biological Sciences |

### Other Experience and Professional Memberships

Member, Students for a National Health Program
Member, Japanese Medical Society of America
Member, Columbia University Neuroscience Outreach

### Honors

| | |
|---|---|
| 2007 | Valedictorian, Steamboat Springs High School |
| 2009 - 2011 | Dean's List, Columbia University |
| 2010 | William B. & Allan Taylor DeVoe Scholarship, Columbia University |
| 2014 - 2015 | Dean's List, Columbia University |
| 2015 | Harry G. DeMeo, M.D. Scholarship, Columbia University |
| 2017 | IFAP Global Health Research Fellowship, Columbia University |
| 2017 | Mitsui USA Research Fellowship, Weatherhead East Asian Institute, Columbia University |

## C. Contribution to Science

1. Physiology and Neurobiology Laboratory, Kyoto University, Kyoto, Japan

During the summer between my 1st and 2nd year in medical school, I traveled to Japan to complete an 8-week rotation in Dr. Tadashi Isa's neurobiology lab. There I had my first opportunity to do experimental work with

nonhuman primates. I was involved in two complementary projects: a behavioral project using a visually guided saccade task and a histological project preparing and examining fixed brain slices.

The lab was working to map the neural circuits that mediate a phenomenon called “blindsight” in macaque monkeys. Blindsight was discovered in the 1970’s in D.B., an epileptic patient who underwent neurosurgery to unilaterally remove portions of his occipital lobe. This effectively lesioned his V1 primary visual cortex, rendering him blind in the contralesional half of his visual field in both eyes. However, he was found paradoxically to have retained some target-finding abilities when presented with stimuli in his visual scotoma. Further investigation into this phenomenon revealed several secondary pathways from the retina that bypass V1 processing. Visual information traveling in these pathways is thought to give rise to physiological and behavioral effects, without leading to the kind of conscious awareness usually associated with seeing.

One of these auxiliary pathways is known as the retinotectal pathway and carries information from the retina to the pulvinar (Pul) nucleus of the thalamus, by way of the superior colliculus (SC). Another is known as the geniculo-extrastriate pathway, which carries retinal information to the koniocellular layer of the lateral geniculate nucleus (LGN), before traveling on to higher cortical areas. Our research over the summer was focused on performing a double-dissociation experiment to determine which of these two pathways was necessary in the performance of target-finding behavior in V1 lesioned animals. Using muscimol, a GABAA agonist, we were able to alternatively inhibit Pul and LGN, and assess our animal’s ability to accurately perform saccades to targets displayed on a computer screen. We found significant inhibition of target-finding behavior with injections into Pul, lending evidence to the importance of the retinotectal pathway. However, we also observed some mild target-finding inhibition following injections into LGN, but we could not be sure whether the weaker LGN response was due to underlying neural connectivity, or whether we needed to further refine our experimental techniques.

The histological work involved preparing and examining brain slices from a prior V1 lesioned animal. Before being sacrificed, this animal received injections into the SC with an anterograde adeno-associated viral vector expressing the marker dsRed. Working from previous descriptions of SC connectivity in the literature, I examined the slides for evidence of any novel neuroplastic changes that might have occurred in response to the V1 lesion. Although this study only concerned a single subject, the staining appeared to show novel connections from the SC to lateral and medial aspects of Pul, where previous descriptions only emphasized connections to inferior Pul – representing a potential anatomical explanation for the retinotectal-mediated target-finding activity.

Following the rotation, I summarized my work in a dedicated lab meeting and presented my findings at a poster conference for Columbia’s Global Health Initiative, which funded my research.

2. Biophysics Laboratory, The Rockefeller University, New York, NY

I worked closely with Prof. Lawrence Sirovich in his lab at The Rockefeller University, where we developed new computational tools for disease prediction using genome-wide association study (GWAS) data. Specifically, we abandoned the standard method of searching for disease-associated loci, which identifies locations with the highest odds-ratio, and instead used an information-theoretical approach. We showed that the odds-ratio approach is biased toward alleles that are very rare but are comparatively less rare in disease versus control cohorts. Information-theory provides a way to perform a least-biased sampling of potentially relevant loci, which avoids the pitfalls associated with using an odds-ratio. We applied our methods to the Fusion Study database, which contained 919 genomic sequences from patients with type-2 diabetes, together with 787 control sequences. We chose this population, because we assumed diabetes to have a strong genetic component, without clear Mendelian inheritance properties.

To perform the analysis, we first split the dataset into two groups, a test and a training set, with roughly equal ratios of cases to controls. Using the training set, we were able to compute the informational complexity associated with each location sampled on the microarray. We then set a cutoff value to select the most informationally salient loci, which differentiated the case patients from the controls. In our final analysis, this step isolated 499 alleles. Using these locations, we were then able to construct a “disease classifier”, which was simply a vector containing the loci in question together with the disease-associated nucleobase found at each loci. This classifier is best conceptualized as a string of 499 symbols, each of which has a genomic “address” that points to the most important locations on the microarray. Using these addresses, we were then able to build similar strings for each subject in the test set, by assembling the symbols found at each address. Finally, we predicted which members of the test set were cases or controls based on how well each subject’s string matched the classifier. Patients whose string most closely matched the classifier string were assumed to have the disease, while patients whose strings differed were assumed to be controls. Using this method, we were able to correctly predict the disease status of the test set at a rate of 75%. While this was a significant result by itself, the loci

associated with the disease classifier unfortunately did not identify any genes that might play a mechanistic role in T2D pathogenesis. However, another exciting application of this approach was that the entire process could be performed in reverse, to create a “control classifier” based on loci that might proffer a protective benefit against the disease in question. Such tools might prove to be useful in researching other weakly-inheritable diseases. Dr. Sirovich published a paper outlining this approach in which he recognized my contributions in the acknowledgments section.

a. Sirovich L. A new structural approach to genomic discovery of disease: example of adult-onset diabetes. Biol Cybern. 2016 Dec;110(6):383-391. PubMed PMID: 27443641.

3. Brain & Mind Research Institute, Weill Cornell Medicine, New York, NY

At the start of my postbac premed program, I volunteered with Dr. Gang Wang, an electrophysiologist in the Iadecola lab at Weill Cornell. There I was first exposed to a variety of basic techniques essential in neurobiology research, such as patch-clamp and field potential recordings, immunocytochemistry, fluorescence microscopy, electrophoresis procedures, calcium imaging, genotyping, and the care and handling of laboratory mice.

I was involved in several projects, the most significant of which was focused on the relationship between Alzheimer’s disease (AD) and homeostatic regulation of body weight. Increased body weight is a known risk factor for developing AD, and accelerated loss of body weight often precedes clinically observable cognitive changes in AD. One of the predominant theories of AD etiology focuses on the extracellular accumulation of amyloid beta (Aβ) proteins, which are thought to lead to neuronal dysregulation. Previously, the lab had demonstrated that GFP-labeled neuropeptide Y (NPY) neurons in the arcuate (Arc) nucleus of the hypothalamus exhibited abnormal electrophysiological responses in mice overexpressing amyloid precursor protein (APP) or in wild-type (WT) brain slices treated with Aβ1-42. To understand what cellular mechanisms might be mediating these changes, we measured membrane potentials and voltage-gated Ca2+ influx. In WT mice, we found that leptin both decreased cytosolic-free Ca2+ and had a hyperpolarizing effect on Arc NPY neurons in vitro, which was consistent with prior knowledge. Next, treatment with Aβ1-42 was found to depolarize those same cells in a dose-dependent manner. We also found that the depolarizing effect of Aβ1-42 was reversed in the setting of nimodipine, an L-type Ca2+ channel blocker. Lastly, nimodipine restored the resting membrane potential in APP mice to that of WT mice. Together, these findings posited a mechanistic explanation of leptin insensitivity in Arc NPY neurons in the setting of AD. We submitted these results as an abstract to the 2015 Society for Neuroscience conference.

a. Wang G, Ishii M, McGuire M, Pensack M, Anrather J, Iadecola C. Voltage-gated Ca2+ influx plays a role in Aβ1-42-induced depolarization of hypothalamic arcuate NPY neurons. Society for Neuroscience; 2015 October 18.

4. Alzheimer’s Disease & Memory Disorders Program, Weill Cornell Medicine, New York, NY

At the start of my postbac premed program, I also started volunteering with Dr. Alon Seifan, a neurologist at Weill Cornell who specialized in Alzheimer’s disease (AD). My clinical responsibilities included taking vitals, administering neuropsychological exams, and helping to manage the daily office operations. My research responsibilities included writing IRB applications, creating and managing the research database, and performing data analysis using R. Our research was based a “comparative effectiveness” approach to disease prevention, which represents a potentially powerful alternative to standard hypothesis-based research. Our project focused on generating a comprehensive online database that collected patients’ baseline health indices, genetic markers, and all prospective medical and lifestyle interventions undertook as part of their routine care. After this data was collected, we planned to work with a team of biostatisticians to analyze the database and discover what factors proved to have the highest association with preventing AD progression.

a. Seifan A, Shih C, Hackett K, Pensack MJ, Schelke MW, Lin M, Patel H, Ganzer CA, Ahmed M, Krikorian R, Tamboer P, Henriquez AM, Isaacson RS, Steinhof S. Detection of neurodevelopmental diversity in memory clinics-Validation of a self-report measure. Res Dev Disabil. 2018 Jun;77:60-67. PubMed PMID: 29660590.
b. Seifan A, Krikorian R, Pensack M, Chen J, Melendez-Cabrero J, Isaacson R. A New Clinical Diagnostic Framework for Assessing Preclinical Alzheimer’s Disease in Healthy At-Risk Adults: A Work In Progress. Clinical Trials on Alzheimer's Disease; 2015 November 04.

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

## Scholastic Performance

| Columbia U. – Columbia College | | |
|---|---|---|
| 2007 | First-Year Scene Lab | A- |
| | Frontiers of Science | A |
| | Literature Humanities I | B |
| | Ballet I | P |
| | General Chemistry I | B |
| 2008 | Rehearsal & Performance I | A |
| | Rehearsal & Performance II | A |
| | Vinyasa Yoga | P |
| | Calculus II | B |
| | Literature Humanities II | B |
| | Intro to Study and Theory of Film | A- |
| | University Writing | B+ |
| | Physics I: Mechanics/ Relativity | B- |
| | Honors Mathematics A | B- |
| | Accelerated Elementary German | A- |
| | Approaches to Poetry | A |
| | Contemporary Civilizations I | A- |
| 2009 | Rehearsal & Performance I | A |
| | Rehearsal & Performance II | A |
| | Honors Mathematics B | B+ |
| | Accelerated Intermediate German | A |
| | Contemporary Civilizations II | A- |
| | Metaphysics | A |
| | Intro to Philosophy of Art | A- |
| | Intro to Higher Mathematics | P |
| | Masterpieces of Western Music | A |
| | Auteur Study: Alfred Hitchcock | A |
| 2010 | Basic Drawing | A |
| | Rehearsal & Performance | A |
| | Acting Lab: Suzuki & Viewpoints | A- |
| | European Social Philosophy | A- |
| | Philosophy and Feminism | B |
| | Major Texts: East Asia | A- |
| | Metaphysics: Ontology of the Arts | A- |
| | Symbolic Logic | B+ |
| | European Phil.: Heidegger | R |
| | Philosophy and History | A- |
| | Masterpieces of Western Art | A |
| 2011 | Acting Lab: Acting the Song | A- |
| | Senior Research: Phil. of Theatre | A- |
| | Kant | A- |
| | Plato | B+ |
| | Shakespeare II | A |
| | Art in China, Japan, and Korea | A- |

| Columbia U. – Postbac. Premed Program | | |
|---|---|---|
| 2014 | General Physics I Lab | A+ |
| | General Physics I | A |
| | General Chemistry I | A+ |
| 2015 | The Science of Psychology | A |
| | General Physics II Lab | A |
| | General Physics II | A+ |
| | General Chemistry II | A+ |
| | General Chemistry Lab | A |
| | Organic Chemistry I Lab | B+ |
| | Organic Chemistry I | B |
| | Biology Lab | A |
| | Biology I | A- |
| 2016 | Organic Chemistry II Lab | B+ |
| | Organic Chemistry II | C |
| | Biology II | B+ |
| **Columbia U. – College of Physicians & Surgeons** | | |
| 2016 | Clinical Gross Anatomy | P |
| | Mech. in Health & Disease | P |
| | Clinical Medicine I | P |
| | Global & Pop. Health | P |
| | Biochemistry | P |
| 2017 | Psychiatric Medicine | P |
| | Clinical Medicine: Tutorials | P |
| | Body in Health & Disease I | P |
| | Clinical Medicine II | P |
| | Clinical Medicine III: Tutorials | P |
| | Body in Health & Disease II | P |
| | Clinical Medicine III | P |
| 2018 | Urology Clerkship | P |
| | Orthopedic Surg. Clerkship | P |
| | OB/GYN Clerkship | HP |
| | Selective: Physical Med./ Rehab. | P |
| | Medicine Clerkship II | HP |
| | Primary Care Clerkship | HP |
| **Columbia U. – Grad. School of Arts & Sciences** | | |
| 2018 | Analysis for Neuroscientists | A+ |
| | Survey of Neuroscience | A |
| 2019 | Crafting a Research Proposal | P |
| | Responsible Conduct of Research | A |
| | Neurobio. II: Dev. & Systems | A |
| | Devices & Analysis for Neuro. | B |
| | Student Journal Club | P |
| 2020 | Intro to Theoretical Neuroscience | P |
| | Building a Specific Aims Page | P |
| | Student Journal Club | P |
| | Mathematical Tools for Theory | P |

From BA Program: P indicates pass. R indicates audit. Minimum passing grade is a D.

From MD Program: P indicates pass. HP indicates high pass. H indicates honors. All classes in 2016-2017, as well as Urology, Orthopaedic surgery, and the Selective in physical medicine & rehabilitation were graded pass/ fail.

From PhD Program: P indicates pass. Minimum passing grade is a D.

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

## BIOGRAPHICAL SKETCH

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

NAME: C. Daniel Salzman

eRA COMMONS USER NAME (credential, e.g., agency login): SALZMAND

POSITION TITLE: Professor

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 |
|---|---|---|---|
| University of Massachusetts, Amherst, MA | B.A. | 05/1985 | Philosophy |
| Stanford University, School of Medicine, CA | M.D. | 06/1995 | Medicine |
| Stanford University, School of Medicine, CA | Ph.D. | 06/1995 | Neuroscience |
| Stanford University Hospital, Stanford, CA | Residency | 06/1999 | Psychiatry |
| Stanford University, Stanford, CA | Postdoc | 08/1999 | Neuroscience |

### A. Research Statement

The Salzman lab investigates emotional and cognitive processes in the brain in non-human primates and mice. In non-human primates, we revealed that neurons in the amygdala represent the positive or negative value of stimuli associated with appetitive or aversive outcomes. In mice, we showed that neural representations of appetitive and aversive unconditioned stimuli mediate both innate and learned valence-specific emotional responses. Given the emphasis on rigorous behavior and quantitative analysis of neural activity in relation to behavior within the work on the primate side of the lab, Max Pensack is in an incredibly rich environment to learn and apply in-depth analysis of behavioral paradigms and neural activity as he pursues his own research path. I have substantial external grant funding from the NIMH, Simons Foundation and other funding sources to support research in in both monkeys and mice. These funding sources will ensure that there will be ample resources to cover all costs of Max's project not covered by this application.

Currently, there are 2 postdoctoral fellows, two graduate students, an Assistant Professor in Psychiatry, and 2 lab technicians who all work in the monkey research arm of the Salzman laboratory. These trainees interact regularly with members of the mouse side of my lab (3 postdoctoral fellows, 1 graduate student, and 2 additional lab technicians), and with other labs at Columbia, primarily the Fusi, Shadlen, Axel and Churchland labs. My trainees additionally benefit from my long history of training graduate students and postdoctoral scientists. In the last 10 years, 8 students have graduated from the lab with a Ph.D. (2 as part of the M.D./Ph.D. program). One of these students is now Director of the Champalimaud Neuroscience Institute in Lisbon (Joe Paton). In addition, four former postdoctoral fellows have become faculty members at academic institutions. I have also served for more than 10 years as Training Director on the postdoctoral NIMH T32 entitled "Research Training in Mood and Anxiety Disorders: from Animal Models to Patients." I am the Director of the Leon Levy Fellowship program at Columbia which supports the career development of M.D., Ph.D. psychiatrists-in-training. I currently either mentor or co-mentor 5 K01, K08, or K99 awardees, and my support of their application process and research development is emblematic of my training experience. Finally, since September 2011, I have chaired the Search Committee for new faculty in the Department of Neuroscience and/or at the Zuckerman Mind Brain Behavior Institute. These experiences provide the perspective needed to advise and mentor postdoctoral fellows and students as they seek to obtain their own independent faculty positions and forge an academic career.

**B. Positions and Honors**

**Positions and Employment**

1986-1987 Research Technician, Dept. of Cell Biology, Albert Einstein College of Medicine
1995-1996 Stanford University Hospital, Intern in Psychiatry
1996-1999 Stanford University Hospital, Resident in Psychiatry
1999-1999 Postdoctoral Fellow, Stanford University, Depts. of Psychiatry and Neurobiology
1999-2000 Research Associate, Stanford University, Depts. of Psychiatry and Neurobiology
2000-2001 Senior Research Scientist, Stanford University, Depts. of Psychiatry and Neurobiology
2001-2007 Assistant Professor in Psychiatry and the Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University
2006-present Research Scientist VII, New York State Psychiatric Institute, Columbia University
2007-2010 Assistant Professor in the Depts. of Psychiatry and Neuroscience, College of Physicians and Surgeons, Columbia University
2010-2014 Associate Professor of Neuroscience in the Depts. of Psychiatry and Neuroscience, College of Physicians and Surgeons, Columbia University
2012-present Co-Director, Mahoney Center for Brain and Behavior Research at Columbia University
2012-present Co-Director, Division of Neurobiology & Behavior at New York State Psychiatric Institute
2014-present Professor of Neuroscience in the Depts. of Psychiatry and Neuroscience, College of Physicians and Surgeons, Columbia University

**Other Experience and Professional Memberships**

1990-present Member, Society for Neuroscience
2002-present American Board of Psychiatry and Neurology (Psychiatry)
2004-2009 Associate Editor, Journal of Neuroscience
2010-2013 Program Committee, Society for Neuroscience
2011-present Editorial Board, Cell Reports
2016-present Associate Editor, Proceedings of the Royal Society B: Biological Sciences

**Honors**

1985 Phi Beta Kappa
1991 Howard Hughes Medical Institute award for return to medical studies
1996 Donald B. Lindsley Prize in Behavioral Neuroscience, given by the Society for Neuroscience
1997 NIMH Outstanding Resident Award
1998 Excellence in Psychiatry Residency Award, presented at the 1998 American Psychiatric Association Annual Conference
1999 Lily Award, presented at the 1999 Society of Biological Psychiatry Annual Meeting
2003 APA/SmithKline Beecham Young Faculty Award for Research Development in Biological Psychiatry
2008 Harold and Golden Lamport Award for Excellence in Clinical Science Research, Columbia University

**C. Contribution to Science**

1) The central challenge of cognitive and affective neuroscience is to understand how complex neural circuitry produces thoughts, feelings, and behaviors that constitute mental life. Early work in my lab challenged the commonly held belief that the amygdala primarily mediates emotional learning and behavior about aversive stimuli. We combined neurophysiological recording in the amygdala of behaving monkeys with appropriately designed tasks that tested whether amygdala neurons process both emotional valences. We discovered that distinct populations of neurons in the amygdala represent the positive and negative value (or valence) of visual stimuli, where positive and negative values refer to the learned association of visual stimuli with rewarding and aversive outcomes, respectively (Paton et al., 2006; Belova et al., 2008). However, amygdala responses to both appetitive and aversive unconditioned stimuli were found to be modulated by expectation of reinforcement (Belova et al., 2007). Some neurons exhibited this property a valence non-specific manner, reflecting the likely role of the amygdala in a range of cognitive and emotional processes, including valence non-specific processes like arousal and attention. In our most recent work, we have discovered that the amygdala, and not OFC and ACC, provides a neural representation of the hierarchical rank of individuals within a social group, and that this representation is provided by neural ensembles that also represent the value of non-social stimuli (Munuera et

al., 2018). These data argue against the strongest version of the Social Brain Hypothesis, the hypothesis that social processing occurs in dedicated neural circuits. The amygdala appears to be a site of convergence for representing the motivational meaning of both social and non-social stimuli.

a) Paton, JJ, Belova, MA, Morrison, SE and Salzman, CD. The primate amygdala represents the positive and negative value of visual stimuli during learning. ***Nature*** 439: 865-70, 2006. PMCID: PMC2396495.
b) Belova, MA, Paton, JJ, Morrison, SE and Salzman, CD. Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. ***Neuron*** 55: 970-984, 2007. PMCID: PMC2042139.
c) Belova, MA, Paton, JJ and Salzman, CD. Moment to moment tracking of state value in the amygdala. ***Journal of Neuroscience*** 28(40): 10023-30, 2008. PMCID: PMC2610542.
d) Munuera, J, Rigotti, M and Salzman CD. Shared neural coding for social hierarchy and reward value in primate amygdala. ***Nature Neuroscience*** 2018 Feb, doi: 10.1038/s41593-018-0082-8. [Epub ahead of print]

2) One of the great challenges in neuroscience is to understand the causal role of neural activity in mediating different aspects of cognition and behavior. Ideally, causal experiments should test whether observed neurophysiological response properties are indeed indicative of the role of those neurons in behavior. The physiological studies described above characterize identified amygdala neurons belonging to appetitive and aversive networks. However, neurons from these two networks appear to be anatomically intermingled despite controversial studies in recent years which we have reviewed (O'Neill et al., 2018). We began to use the mouse preparation to investigate the causal role of appetitive and aversive circuits in the amygdala in emotional behavior, before any molecular marker specific for appetitive or aversive networks had been identified. In particular, we devised a method for identifying, marking, and manipulating ensembles of neurons in the amygdala based on their physiological response properties. We recently showed that neural representations of appetitive and aversive unconditioned stimuli mediate both innate and learned valence-specific emotional responses (Gore et al., 2015a). These findings were also reviewed in (Gore et al., 2015b), where we discuss the challenges and accomplishments associated with performing causal experiments by manipulating the activity of physiologically classified neurons.

a) Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA, Salzman CD*, Axel R*. Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. ***Cell*** 2015 Jul 2; 162(1): 134-45. PMCID: PMC4526462. *co-senior/corresponding author
b) Gore F, Schwartz EC, Salzman CD. Manipulating neural activity in physiologically classified neurons: triumphs and challenges. ***Philosophical Transactions of the Royal Society London B: Biological Sciences***. 2015 Sep 19; 370(1677). Review. PMCID: PMC4528828.
c) O'Neill PK, Gore F, Salzman CD. Basolateral amygdala circuitry in positive and negative valence. ***Current Opinion in Neurobiology***. 2018 Apr; 49: 175-183. PMCID: 29525574.
d) Wang L, Gillis-Smith S, Peng Y, Zhang J, Chen X, Salzman CD, Ryba N, Zuker C. The coding of valence and identity in the mammalian taste system. ***Nature***. 2018; 558: 127-131. PMCID: 29849148.

3) Scientists have long appreciated that emotional processes direct cognitive resources towards stimuli that promote or threaten survival. This preferential processing requires that a threatening or rewarding stimulus be identified and located in the environment to guide spatial attention. It had commonly been believed that the amygdala registers the motivational significance of such stimuli, but that the amygdala did not mediate the second step, the guidance of spatial attention. This view derives from the fact that, in primates, the densest visual inputs to the amygdala originate from visual areas involved in object recognition rather than spatial localization. Consequently, it has been assumed that the amygdala could regulate non-spatial processes like arousal but not spatial attention. We tested specifically whether this assumption is true, i.e. whether the amygdala is involved in the allocation of spatial attention to motivationally relevant stimuli (Peck et al., 2013). We recorded the activity of single amygdala neurons from monkeys performing an attentionally demanding task in which motivationally significant visual cues biased monkeys' spatial attention. Surprisingly, amygdala activity was spatially selective, with individual neurons combining information about the spatial location and motivational significance of visual stimuli. Moreover, fluctuations in amygdala activity predicted variations in the monkeys' trial-to-trial allocation of spatial attention. These data highlight a novel role for the amygdala in directing spatial attention to motivationally meaningful stimuli, which may explain the role of dense feedback projections from the amygdala to visual cortical areas. Subsequent papers in the lab have established that 1) amygdala neural activity

reflects spatial attention not only towards stimuli promising rewards but also to stimuli threatening the possibility of punishment (Peck and Salzman, 2014a; and 2) spatial processing is also present in a major target structure of the amygdala that may mediate attentional behavior, the basal forebrain (Peck and Salzman, 2014b). Additionally, we have studied how reward expectation modulates visual representations in V4, and how these modulations may underlie the perceptual benefits of attention. Of course, the amygdala projects directly to V4, and it may also influence V4 activity indirectly via the basal forebrain. We demonstrate that increased signal-to-noise in the representation of V4 does not confer the benefits of attention; instead, these benefits appear to derive from downstream selection mechanisms (Baruni et al., 2015).

a) Peck, CJ, Lau, B and Salzman CD. The primate amygdala combines information about space and value. ***Nature Neuroscience*** 2013 Mar, 16(3): 340-8. PMCID: PMC3596258.
b) Peck CJ and Salzman CD. The amygdala and Basal forebrain as a pathway for motivationally guided attention. ***Journal of Neuroscience*** 2014 Oct 8; 34(41): 13757-67. PMCID: PMC4188973.
c) Peck CJ and Salzman CD. Amygdala neural activity reflects spatial attention towards stimuli promising reward or threatening punishment. ***eLife*** 2014 Oct 30; 3. 10.7554/eLife.04478. PMCID: PMC4238057.
d) Baruni JK, Lau B, and Salzman CD. Reward expectation differentially modulates attentional behavior and activity in visual area V4. ***Nature Neuroscience*** 2015 Nov; 18(11): 1656-63. PMCID: PMC4624579.

4) Interactions between prefrontal cortex and the amygdala may underlie flexible emotional behavior which may be described as involving the cognitive regulation of emotion. Following initial work using reversal learning to study how the emotional or motivational significance of stimuli may be updated, (Morrison et al., 2011), recent work has extended this work to examine other factors other than how changing reinforcement contingencies causes changes in neural activity. For example, we recently examined how reward history is differentially tracked by the amygdala and OFC to update representations of reinforcement expectation, which is a type of flexible cognitive regulation (Saez et al., 2017). Most recently, we have turned to serial reversal learning tasks in which monkeys learn to use their knowledge of the temporal statistics of event to adjust behavior using inference. These temporal statistics might comprise an abstract (un-cued) context, which can be used to predict reinforcement more accurately (Saez et al., 2015). Neurons in the amygdala, OFC, and ACC represent this abstract context. The representation of the abstract context was demonstrated to reflect the learned set of CS-US contingencies that defined a “task set”. In other words, monkeys abstracted the task set, which characterized the abstract context, and neural representations reflected this process of abstraction. Our work on abstraction most recently has investigated brain areas likely responsible for the formation of abstract representations (hippocampus) and their utilization to mediate flexible behavior (PFC). An integral part of this study of abstract representations has been work with Stefano Fusi that develops a new conceptual framework and analytic methodology for characterizing neural representations that focuses on how the geometry of neural representations can represent variables to support different forms of flexible cognitive and emotional behavior (Bernardi et. al., 2020).

a) Bernardi S, Benna MK, Rigotti M, Munuera J, Fusi S, Salzman CD. The Geometry of Abstraction in the Hippocampus and Prefrontal Cortex. ***Cell*** 2020 Nov12;183(4):954-967. PMID: 33058757.
b) Morrison, SE, Saez A, Lau B and Salzman CD. Different time courses for learning-related changes in amygdala and orbitofrontal cortex. ***Neuron*** 2011 Sep 22, 71(6): 1127-40. PMCID: PMC3236094.
c) Saez A, Rigotti M, Ostojic S, Fusi S, Salzman CD. Abstract Context Representations in Primate Amygdala and Prefrontal Cortex. ***Neuron*** 2015 Aug 19; 87(4): 869-81. PMCID: PMC4574873.
d) Saez RA, Saez A, Paton JJ, Lau B, Salzman CD. Distinct Roles for the Amygdala and Orbitofrontal Cortex in Representing the Relative Amount of Expected Reward. ***Neuron*** 2017 Jul 5;95(1): 70-77. PMCID: PMC5710843.

5) My work as a student remains extremely well known in the field, and it helped catapult my subsequent research career. I employed microstimulation combined with recording techniques to manipulate selectively the activity of physiologically characterized direction-selective neurons in visual area MT while monkeys performed a motion discrimination task. We provided causal evidence linking the activity of direction selective neurons to perceptual judgments of motion direction. These studies have been widely cited in many papers and textbooks, and they are often still taught in graduate classes on the neurobiology of visual perception and decision-making.

a) Salzman, CD, Britten, KH, and Newsome, WT. Cortical microstimulation influences perceptual judgments of motion direction. ***Nature*** 346: 174-177, 1990. PMID: 2366872.

b) Salzman, CD, Murasugi, CM, Britten, KH and Newsome, WT. Microstimulation in visual area MT: effects on direction discrimination performance. ***Journal of Neuroscience***. 12(6): 2331-2355, 1992. PMID: 1607944.
c) Salzman, CD and Newsome, WT. Neural Mechanisms for Forming a Perceptual Decision. ***Science*** 264: 231-237, 1994. PMID: 8146653.

**Complete List of Published Work in MyBibliography:**
**http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40348001/?sort=date&direction=descending**

**D. Research Support**

**Ongoing Research Support**

Columbia Boehringer-Ingelheim Collaboration Leibel (PI) 11/1/2020-10/31/2021
Role: Co-I

NIMH R01 MH082017 Salzman (PI) 4/10/2008-10/31/2022
Neurophysiology underlying neural representations of value
This study examines the amygdala in relation to OFC during different tasks involving reinforcement learning.

Simons Foundation: Collaboration on the Global Brain Fusi (PI) 7/1/2017-6/30/2022
Neural mechanisms of context dependent cognitive behavior
This study utilizes mathematical techniques including dimensionality reduction, state space analyses, and neural network modeling to analyze neural data so as to determine how contexts affect learning and decision-making. Role: Co-I

NIMH T32 MH015144 Roose (PI) 7/1/1978-6/30/2024
Research Training in Mood and Anxiety Disorders: From Animal Models to Patients
The primary goal of this project is to train postdoctoral fellows for careers as independent researchers in Affective, Anxiety and Related Disorders.
Role: Training Director

NIDA R21 DA045989 Salzman (PI) 9/30/2018-8/31/2021
Neurophysiological mechanisms underlying rTMS treatment of addiction
This study probes the mechanisms by which repetitive transcranial magnetic stimulation (rTMS) reduces cocaine craving, by assessing the effects on neural activity in the prefrontal cortex and amygdala.

NIMH R21 MH116348 Salzman (PI) 12/24/2018-11/30/2021
The interactive roles of the amygdala and orbitofrontal cortex during reversal learning.
This grant investigates the dynamic interactions between orbitofrontal cortex and the amygdala during a reversal learning task involving both rewarding and aversive outcomes.

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

## BIOGRAPHICAL SKETCH

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

NAME: Fusi, Stefano

eRA COMMONS USER NAME (credential, e.g., agency login): SF2237

POSITION TITLE: Professor of Neuroscience

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | Completion Date | FIELD OF STUDY |
|---|---|---|---|
| University of Rome, La Sapienza | Laurea | 07/1992 | Physics |
| Hebrew University of Jerusalem | Ph.D. | 09/1999 | Physics |

**A. Personal Statement**

The recorded neural responses are very diverse and sometimes not easily interpretable. We developed a theoretical model inspired by machine learning studies that shows that this diversity plays a fundamental role in the dynamics of the neural circuits, especially when multiple sources of information (e.g. internally represented context and sensory stimuli) have to be integrated together (Rigotti et al., Frontiers in Computational Neuroscience, 2010; Rigotti et al., Neuroimage, 2010; Barak et al., J. Neuroscience, 2013). The theory provides guidelines to analyze and decode the recorded neural activity and it predicts that mixed selectivity (selectivity to multiple task relevant aspects) is a fundamental property of neuronal responses. In particular, mixed selectivity neurons not only could be as informative as highly specialized cells, but in complex tasks they may be the most important cells for driving the dynamics of the local circuit. We validated these predictions by analyzing the neural activity recorded in pre-frontal cortex (Rigotti et al. Nature, 2013) and by showing that the dimensionality of the neural representations is maximal, as suggested by the presence of mixed selectivity neurons. Studying high dimensional representations require new analytical tools that take into consideration the fact that the information about the task relevant variables is distributed across multiple neurons. For this reason, in all the experiments we recently analyzed we developed techniques to decode task relevant variables from the activity of populations of neurons (Rigotti et al., Nature 2013; Spellman et al. Nature 2015; Saez et al. Neuron 2015). Recently, we extended these techniques to determine whether abstract variables like context are represented at the population level. In a new theoretical framework, we constructed neural representations of multiple abstract variables and we developed data analysis techniques that look at the geometry of the neural representations to assess whether variables are represented in an abstract format (Bernardi et al. biorxiv 2018).

My background is theoretical physics, but I have been working on theoretical neuroscience for over 25 years and I have gained a great deal of experience on neural network theory, electrophysiology experiments, data analysis and modeling. I did experiments myself during my PhD at the Hebrew University of Jerusalem (Amit, Fusi, Yakovlev, Neural Computation, 1997; Yakovlev et al 1998, Nat. Neurosci.), and recently I have been working on collaborative projects with Dr. E.K. Miller at MIT (Fusi et al 2007, Neuron, Rigotti et al., Nature, 2013), with Dr. Salzman (Rigotti et al. 2010, Neuroimage, Saez et al. 2015, Neuron) on experiments on pre-frontal cortex and amygdala of behaving monkeys, and with Dr Gordon on the interaction between hippocampus and pre-frontal cortex (Spellman et al. 2015, Nature) and with Dr. Hen on the neural representations of space in the hippocampus. I am currently working at the Center for Theoretical Neuroscience, where my students, postdocs, and I greatly benefit from the continuous interactions with the other investigators at the Center directed by Drs. L. F. Abbott and K. Miller. The Center provides a thriving intellectual environment with, at the present time, 11 outstanding postdoctoral researchers and 15 fulltime and rotation graduate students, in addition to 5 faculty and extended visitors. In my academic career I have mentored 6 PhD students and 6 Postdocs. Four of them are currently holding a Faculty position (Stony Brook, Technion (Israel), Centre de ricerca matematica (Barcelona), École normale supérieure (Paris) and two a full-time independent researcher position in private companies (IBM and Intel).

## B. Positions and Honors

**Positions and Employment:**

1993-1995 INFN (National Institute for Nuclear Physics) Fellow at ISS (Italian NIH), Italy
1995-1999 INFN Researcher (Italian "Ricercatore") at the University of Rome La Sapienza, Italy
1999-2005 Research Assistant at the Institute of Physiology, University of Bern, Switzerland
2003-2004 Visiting Scientist at Brandeis University, MA
2005-2009 Assistant Professor at the Institute for Neuroinformatics, ETH, Zurich, Switzerland
2005-2007 Senior Research Associate, Columbia University, NY
2007-2009 Assistant Professor, Dept. of Neuroscience, Columbia University, NY
2009-2019 Associate Professor, Dept. of Neuroscience, Columbia University, NY
2019- Professor, Dept. of Neuroscience, Columbia University, NY

**Professional Memberships:**

2003 - Member, Society for Neuroscience

**Honors:**

1990 E. Persico prize (Accademia dei Lincei), Rome, Italy

## C. Contribution to Science

1. The complexity and diversity of the numerous biological mechanisms that underlie memory is both fascinating and disconcerting. The molecular machinery that is responsible for memory consolidation at the level of synaptic connections is believed to employ a complex network of highly diverse biochemical processes that operate on different timescales. Understanding how these processes are orchestrated to preserve memories over a lifetime requires guiding principles to interpret the complex organization of the observed synaptic molecular interactions and explain its computational advantage. In the last 25 years I have been working on synaptic models that can efficiently harness biological complexity to store and preserve a huge number of memories on long timescales. The theoretical framework that I developed has been applied to a large number of memory systems and it helped to identify the computational principles that underlie memory consolidation, both at the level of individual synapses and at the system level.

a. Fusi S, Drew PJ, Abbott LF. Cascade models of synaptically stored memories. *Neuron*. 2005 Feb 17; 45(4):599-611. PubMed PMID: 15721245.
b. Fusi S, Asaad WF, Miller EK, Wang XJ. A neural circuit model of flexible sensorimotor mapping: learning and forgetting on multiple timescales. *Neuron*. 2007 Apr 19; 54(2): 319-33. PMCID: PMC2833020.
c. Benna M and Fusi S. 2016. Computational Principles of biological memory, *Nature Neuroscience*, 19, 1697–1706 (2016)
d. Iigaya, K., Sugrue, L., Corrado, G.S., Loewenstein, Y., Newsome, W.T. and Fusi, S., 2019. Deviations from the matching law reflect reward integration over multiple timescales. *Nature Communications*, in press.

2. Electronic neuromorphic machines combine the architectures and computational principles of biological systems with the power of semiconductor electronics. The resulting systems can learn autonomously to solve real-world problems in complex, changing environments, consuming only a small fraction of the energy required by more conventional electronic devices. For these reasons the neuromorphic approach has recently received much attention from the scientific community, the industry (IBM, HP and HRL) and the major funding agencies (Human Brain Project, DARPA SyNAPSE project). Most neuromorphic devices suffer from major limitations in their memory capacity. I discovered these limitations at the beginning of the nineties, working with D. Amit, and together we understood that the limitations derive from the simplicity of the switch-like mechanisms used for implementing plastic synapses. This simplicity not only contrasts with the complexity of biological synapses, but also makes large scale neuromorphic devices highly inefficient. Guided by studies in experimental neuroscience (see 1), I developed synaptic models that solve this memory capacity problem. These studies form the basis of a new generation of scalable neuromorphic devices that are currently being developed.

a. Amit DJ and Fusi S. Learning in neural networks with material synapses. *Neural Computation* 1994 6(5): 957-982.
b. Fusi S, Annunziato M, Badoni D, Salamon A, Amit DJ. Spike-driven synaptic plasticity: theory, simulation, VLSI implementation. *Neural Computation* 2000 Oct; 12(10): 2227-58. PMID: 11032032.
c. Brader JM, Senn W, Fusi S. Learning real-world stimuli in a neural network with spike-driven synaptic dynamics. *Neural Computation*. 2007 Nov; 19(11): 2881-912. PMID: 17883345.
d. Martí D, Rigotti M, Seok M, Fusi S. 2016. Energy-efficient neuromorphic classifiers. *Neural Computation*, 2016, Vol. 28, No. 10, Pages: 2011-2044

3. The majority of neurons, especially in higher-order brain structures like the prefrontal cortex, often exhibit complex and diverse response properties that are not organized anatomically, and that simultaneously encode different parameters. These neurons are said to have mixed selectivity to multiple aspects of the task. The theory of neural networks and machine learning predicts that such diverse mixed selectivity neurons should play an important role in complex cognitive tasks that require the integration of multiple variables. A quantitative measure of such “mixing” and “diversity” that correlates with the performance of the cortical circuit is the dimensionality of the neural representations. The dimensionality of a neural representation is a property of a set of vectors, each of which represents the firing rates of N neurons in a distinct (experimental) condition. The pattern of activity encoded in each such vector can be thought of as a point in an N-dimensional space. Over a set of such points, the dimensionality is defined as the minimal number of coordinate axes needed to specify the position of all the points. I recently demonstrated in behaving monkeys that the dimensionality of a representation in dorsolateral pre-frontal cortex is maximal, reflecting the mixed selectivity properties of individual neurons, and the dimensionality predicted cognitive performance on a complex task (Rigotti et al. Nature 2013; Fusi et al. 2016; Lindsay et al. 2017), proving that the diversity of the neural response properties is important for performing complex tasks. This work and his future extensions have been inspired by my theoretical studies on neural networks (see e.g. Rigotti et al. 2010; Barak et al 2013).

a. Rigotti M, Barak O, Warden MR, Wang XJ, Daw ND, Miller EK, Fusi S. The importance of mixed selectivity in complex cognitive tasks. *Nature*. 2013 May 30; 497(7451): 585-90. PMCID: PMC4412347.
b. Saez A, Rigotti M, Ostojic S, Fusi S, *Salzman CD.* Abstract Context Representations in Primate Amygdala and Prefrontal Cortex*. Neuron*. 2015 Aug 19; 87(4): 869-81. PMCID: PMC4574873.
c. Lindsay, G. W., Rigotti, M., Warden, M. R., Miller, E. K., & Fusi, S. (2017). Hebbian Learning in a Random Network Captures Selectivity Properties of the Prefrontal Cortex. Journal of Neuroscience, 37(45), 11021-11036.
d. Bernardi, S., Benna, M.K., Rigotti, M., Munuera, J., Fusi, S. and Salzman, D., 2018. The geometry of abstraction in hippocampus and prefrontal cortex. bioRxiv, p.408633.

**Complete List of Published Work in Google Scholar:**
**https://www.ncbi.nlm.nih.gov/myncbi/stefano.fusi.1/bibliography/public/**

**D. Research Support**

ACTIVE

5R21NS108380-02 (Badre) 08/01/2018 – 07/31/2020
NIH/NINDS
Mapping representational format across the human brain
Goals: To estimate the dimensionality of neural representations using fMRI. The original idea of using repetition suppression to estimate dimensionality is based on our theoretical work and we will work together with the team at Brown to test this idea in a fMRI experiment.
Role: Subaward Co-I

HR0011-18-2-0025 (Tolias) 02/09/2018 – 02/08/2022
DARPA L2M
Continual learning across synapses, circuits, and brain areas

Goals: To develop theoretical frameworks for understanding the computational principles behind the brain's ability to learn continuously and principles to advance machine learning.
Role: Subcontract Co-PI

DBI-1707398 (Abbott) 08/01/2017 – 07/31/2021
NSF NeuroNex Theory Team
Columbia University Theoretical Neuroscience Hub
Goals: Provide theoretical support and guidance through all stages of discovery in Neuroscience
Role: Co-PI

542983SPI (Fusi) 07/01/2017 – 06/30/2022
Simons Foundation
Neural mechanisms of context dependent cognitive behavior
Understanding the mechanisms that underlie the learning and utilization of knowledge of contexts to guide decision-making.
Role: PI

5R01NS094659-05 (Bruno) 07/15/2016 – 04/30/2021
NIH/NIMH
The behavioral functions of upper and lower cortical layers
The major goals of this project are identifying the contributions of specific cortical layers to normal circuit function to allow for better treatment of pathological conditions.
Role: Co-I

5R01MH082017-12 (Salzman) 04/10/2008 – 10/31/2022
NIH/NIMH
Neurophysiology underlying neural representations of value
The major goals of this project are to understand interactions between orbitofrontal cortex and the amygdala in determining the value of visual stimuli.
Role: Co-I

1R01NS113078-01 (Goldberg, Strick, Fusi) 08/01/2019 – 04/30/2024
NIH/BRAIN Initiative
The cerebro-cerebellar-basal-gangliar network for visuomotor learning
This proposal tests the hypothesis that the brain accomplishes visuomotor associative learning using an anatomically defined closed-loop network, including the prefrontal cortex, the basal ganglia, and the cerebellum.
Role: MPI

ADRG (Hussaini) 07/01/2019 – 06/30/2022
Brightfocus
Does Pathology in Locus Coeruleus Trigger AD?
Goal: The locus coeruleus (LC) of the brain is important for sleep and memory, and has been shown to be the first region to contain a bad protein called tau in their neurons, which causes Alzheimer's disease. By studying electrical activity of LC neurons in animals performing memory tasks and during sleep, we will find out if tau is preventing LC to function normally, and causes sleep and memory problems. In addition, we will make LC neurons sensitive to light, so that we can control their activity by shining light and see if this restore its function and reverse sleep and memory problems.
Role: Co-I

1R01MH120292-01 (Siegelbaum) 08/26/2019 – 05/31/2024
NIH/NIMH
Regulation of social aggression through hippocampal CA2 inputs to lateral septum
The goal of this project is to characterize CA2 circuits and neural activity during aggressive and non-aggressive social exploration in mice.
Role: Co-I

1R01NS113113-01 (Shadlen) 07/15/2019-03/31/2024
NIH/NINDS
Computational and circuit mechanisms of decision making
The goal of this project is to leverage knowledge of visual processing by the brain to reveal how the brain makes
increasingly complex decisions
Role: Co-I

1R21AG066168-01 (Hussaini) 09/15/2019 – 05/31/2021
NIH/NIA
Decoding Early Signs of Alzheimer’s Disease in The Lateral Entorhinal Cortex Using Machine Learning
The major goal of this project is to identify early signs of neuronal dysfunction in the lateral entorhinal cortex, which is selectively vulnerable in Alzheimer's disease.
Role: Co-I

R01AG062259 (PI: Hussaini) 04/01/2020 – 03/31/2025
NIH/NIA
Electrophysiological Evaluation of Brain Regions Vulnerable to Alzheimer’s Disease
The major goal of the project is to identify how vulnerable brain regions are affected by tau and abeta pathology. We will use in vivo electrophysiology and optogenetic methods combined with machine learning approach to identify and reverse neuronal dysfunction.
Role: Co-I

# PHS Fellowship Supplemental Form

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

## Introduction

1. Introduction to Application
(for Resubmission applications)

## Fellowship Applicant Section

| | |
|---|---|
| 2. Applicant's Background and Goals for Fellowship Training* | MJP_F31_Background_Goals.pdf |

## Research Training Plan Section

| | |
|---|---|
| 3. Specific Aims* | Specific_Aims.pdf |
| 4. Research Strategy* | RS.pdf |
| 5. Respective Contributions* | MJP_F31_Respective_Contributions.pdf |
| 6. Selection of Sponsor and Institution* | MJP_F31_Selection_Sponsor_Institution.pdf |
| 7. Progress Report Publication List (for Renewal applications) | |
| 8. Training in the Responsible Conduct of Research* | F31_RCR_Refresher_updated.pdf |

## Sponsor(s), Collaborator(s) and Consultant(s) Section

| | |
|---|---|
| 9. Sponsor and Co-Sponsor Statements | MJP_F31_Sponsor_Statement.pdf |
| 10. Letters of Support from Collaborators, Contributors and Consultants | |

## Institutional Environment and Commitment to Training Section

| | |
|---|---|
| 11. Description of Institutional Environment and Commitment to Training | MJP_F31_Institution_AY.pdf |
| 12. Description of Candidate's Contribution to Program Goals | |

## Other Research Training Plan Section

### Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☑ Yes ☐ No

| | | |
|---|---|---|
| 13. Are vertebrate animals euthanized? | ☑ Yes | ☐ No |
| If "Yes" to euthanasia<br>Is method consistent with American Veterinary Medical Association (AVMA) guidelines? | ☑ Yes | ☐ No |
| If "No" to AVMA guidelines, describe method and provide scientific justification | | |
| 14. Vertebrate Animals | MJP_F31_Vertebrate_Animals.pdf | |





Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T16:53:51.000-05:00

# PHS Fellowship Supplemental Form

**Other Research Training Plan Information**

15. Select Agent Research

16. Resource Sharing Plan MJP_F31_Resource_Sharing.pdf

17. Authentication of Key Biological and/or Chemical Resources

**Additional Information Section**

**18. Human Embryonic Stem Cells**

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), using the registry information provided within the agency instructions. 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):

| | | | |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

19. Alternate Phone Number:

20. Degree Sought During Proposed Award:

| Degree: | If "other", indicate degree type: | Expected Completion Date (MM/YYYY): |
|---|---|---|
| PHD: Doctor of Philosophy | | 09/2023 |

21. Field of Training for Current Proposal*: 160 Neurosciences & Neurobiology

22. Current or Prior Kirschstein-NRSA Support?* ☐ Yes ☑ No

*If yes, identify current and prior Kirschstein-NRSA support below:*

| Level* | Type* | Start Date (if known) | End Date (if known) | Grant Number (if known) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

23. Applications for Concurrent Support?* ☐ Yes ☑ No

*If yes, describe in an attached file:*

24. Citizenship*

U.S. Citizen U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen ☐ With a Permanent U.S. Resident Visa

☐ With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: ☐

Name of Former Institution:*

25. ☐ Change of Sponsoring Institution

# PHS Fellowship Supplemental Form

## Budget Section

**All Fellowship Applicants:**

26. Tuition and Fees*:

☐ None Requested ☑ Funds Requested

| | |
|---|---|
| Year 1 | $50,495.00 |
| Year 2 | $52,010.00 |
| Year 3 | $53,570.00 |
| Year 4 | $0.00 |
| Year 5 | $0.00 |
| Year 6 (when applicable) | $0.00 |
| **Total Funds Requested:** | $156,075.00 |

**Senior Fellowship Applicants Only:**

| | Amount | Academic Period | Number of Months |
|---|---|---|---|
| 27. Present Institutional Base Salary: | | | |

28. Stipends/Salary During First Year of Proposed Fellowship:

| | Amount | Number of Months |
|---|---|---|
| a. Federal Stipend Requested: | | |

| | Amount | Number of Months |
|---|---|---|
| b. Supplementation from Other Sources: | | |

Type (e.g.,sabbatical leave, salary)

Source

## Appendix

**29. Appendix**





Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T16:53:51.000-05:00

## APPLICANT BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

### A. Doctoral Dissertation and Research Experience

"What is the meaning of this behavior?", my professor asked, raising her hand to show a palm to the classroom. I was attending an introductory course on the philosophy of art, and I instantly recalled how a week earlier, in a midtown acting studio, my scene director had posed the same question in the same way. The question was simple enough, but quickly gave way to a host of related concerns: For whom? To what end? In what context? In what culture? What caused the behavior? Now my lab, I hear echoes of these questions daily. One way of charting my path from the arts and humanities to medicine and neuroscience is through my various attempts to understand this question, move closer to it, and at times contribute partial answers.

Behavior is a window into the human condition, and as an undergraduate I was taken in by the range of philosophical attempts to explain that condition. At the same time, I was exploring behavior from experiential and artistic perspectives through my involvement in the theater. These two early loves first introduced me to a cast of overlapping questions that have come to motivate my neuroscientific research, such as the nature of thoughts, feelings, representations, and the mind-body-world relationship. After college, I spent a year pursuing acting professionally in New York, followed by a two-year experience teaching English in southwestern Japan. In Japan, I adjusted to a very different way of life, and saw firsthand how powerfully social dimensions influence our experiences. I wanted to understand all of these issues at a deeper, scientific level and I also wanted to be of service to society, so I decided to go back to school to pursue a career in medicine and to study the neural basis of experience and behavior.

I first joined Dr. Constantino Iadecola's lab at Weill Cornell Medical College, where I conducted research for one year under the supervision of Dr. Gang Wang, the in-house electrophysiologist for the lab. I was attracted to this lab because they studied mouse models of Alzheimer's disease (AD), a subject at the intersection of biological, psychological, and social understandings of health and illness. I worked with Dr. Wang conducting *in vitro* electrophysiology on brain slices from mice exhibiting the Swedish mutation in the APP gene, a well-known genetic variant associated with familial AD. Learning the patch-clamp technique was thrilling; I was astounded by our ability to target a single cell at the tip of my microelectrode, and I can easily recall the excitement I felt on first seeing and hearing the bursting of action potentials. We were interested in understanding how the hypothalamic regulation of body weight becomes altered in the setting of that disease. In human patients and in animal models, increased body weight is a risk factor in developing AD, and once symptomatic, subjects experience stereotypical weight loss as the disease progresses. Previous work had suggested that leptin insensitivity in the arcuate nucleus of the hypothalamus might be mediating these effects. To test this, we conducted single-cell patch-clamp and calcium-imaging experiments using brain slices from WT and APP mice. We found that in APP mice, or in WT mice treated with extracellular amyloid protein, leptin insensitivity was marked by a depolarization of neuropeptide Y neurons in the arcuate, resulting in a significant increase in cytosolic free-calcium. Both of these effects could be reversed by applying the L-type calcium channel blocker nimodipine, suggesting a role for these channels in mediating the metabolic changes seen in AD. While still in the lab, I helped to submit these results as an abstract to the 2015 Society for Neuroscience conference. In addition to gaining skills related to slice electrophysiology and calcium imaging, I was also able to gain exposure to a variety of essential molecular biological techniques during my time in the lab, such as immunocytochemistry, fluorescence microscopy, electrophoresis procedures, genotyping, and the care and handling of laboratory mice. It was a perfect introduction to the world of neuroscience, and encouraged me to continue my path in research.

After working in AD research for over a year (including clinical research in Cornell's department of neurology), I felt that I needed to pursue an experience that would allow me to explore more quantitative and computational approaches to the brain. Through my reading, I came across a fascinating article about facial recognition from Dr. Winrich Freiwald's group at the Rockefeller University, so I contacted him straightaway to see if I could work in his lab. Dr. Freiwald was extremely friendly and enthusiastic, but unfortunately was not looking for research assistants at that time. However, he was generous enough to introduce me to Dr. Lawrence Sirovich, an applied mathematician at Rockefeller, who had previously done interesting work in computational neuroscience, but was currently working in the area of disease prediction using genomic data sets. I met with Dr. Sirovich and his fellow investigator Bruce Knight, both of whom are first-rate scientists and very colorful characters. I learned about their collaboration in Nobel-laureate Keffer Hartline's lab, where they modeled inhibitory network effects in the compound eye of the horseshoe crab *Limulus*, as well as Dr. Sirovich's pioneering contributions to computational face recognition with his 1987 paper using principal component analysis to extract 'eigenfaces' as a basis set of

facial features. Mr. Knight amazingly forwent graduate training in physics, and instead moved from his undergraduate studies directly into a decade-long research position at Los Alamos National Laboratory before becoming a principal investigator in his own right. I knew immediately that I would have a lot to learn from these inspirational figures, and was extremely excited to land the job. As expected, Dr. Sirovich proved to be a terrific mentor to me during the nine months leading up to my first semester in medical school. Through our work, I was exposed to a whole array of important concepts in linear algebra, information theory, and dynamical systems, all of which are standard and powerful tools in scientific modeling. In particular, we were attempting to develop computational methods to extract predictive feature vectors from genome-wide association study (GWAS) datasets. We use type II diabetes as a test-case because the complicated relationship between genetic and environmental etiologies of that disease make it particularly difficult to predict. Prior work on GWAS-based disease prediction usually relied on odds-ratio measures to individually identify the most important genetic loci contributing to the disease. However, these approaches often identified extremely rare mutations as major contributors to the disease, simply because those mutations were slightly more common in the disease cohort than in the control cohort. Instead, we used an information theoretical approach to characterize the dataset as a whole, allowing us to compare the contribution of multiple loci simultaneously to the disease status. I assisted Dr. Sirovich in drafting the manuscript of our work, in which he recognized my contributions in the acknowledgement section. From my experience working with Dr. Sirovich and Mr. Knight, I started to see how powerful quantitative approaches can be in dissecting challenging biological problems, and I started to develop a sense of pride in offering my modest contributions to the scientific tradition as a whole.

I left Dr. Sirovich's lab in fall of 2016 excited to start my clinical training, but with a sense of regret at the prospect of leaving research for an indeterminate number of years, especially as I had yet to apply my newfound enthusiasm for quantitative methods to problems in neuroscience. This feeling motivated me to seek advice from a program director at Columbia's combined MD/PhD program. She encouraged me to explore labs at Columbia where I might like to do doctoral research, but she warned that it would likely be impossible for me to transfer into the NIH-funded dual-degree program. Nonetheless, I sought out labs in different areas of neuroscience in an attempt to make the most informed decision about my research interests. I began in the lab of Dr. Chaolin Zhang, a computational biologist in Columbia's Motor Neuron Center. His lab focuses on the regulation of RNA splicing in the nervous system by modeling interactions between RNA molecules and RNA-binding proteins that make-up and recruit members of the spliceosome. For the remainder of the fall semester, I shadowed one graduate student in the Zhang lab and attended their weekly lab meetings. Although this work was very interesting, by the end of the semester I wanted to explore labs that could offer me a combination of behavioral analysis, electrophysiology, and computational modeling, as these areas had been most exciting for me up to that point. For this reason, I started shadowing a graduate student in Dr. Nate Sawtell's lab, which focuses on noise suppression in circuits of the cerebellum and cerebellar-like structures. Originally Dr. Sawtell's lab used the weakly electric African river fish mormyrid for this work, as that animal must continuously monitor and suppress self-generated electrical signals that it detects with its electrical sensory organ. When I joined the lab, however, Dr. Sawtell had expanded his research program to study the same process in auditory signals recorded from the dorsal cochlear nucleus of the mouse. Although I was extremely busy with my coursework in medical school, I nonetheless came to the lab on the weekends, where I was able to learn how to perform mouse surgeries, set-up automated experiments with microcontrollers, and take my first *in vivo* electrophysiological recordings. I was very excited about doing this work, in part because I hoped eventually to benefit from Dr. Sawtell's long-standing collaboration with Dr. Larry Abbott, who headed the Center for Theoretical Neuroscience at Columbia.

I felt at the time that I might be interested in joining the Sawtell lab, and I started making plans to conduct an independent research project the following summer. However, these plans were eventually sidelined when I suddenly received the opportunity to travel to Japan to conduct research in a non-human primate lab. This opportunity came from one of my medical school instructors, Dr. Takeshi Sakurai, who had also been giving me guidance on how to best pursue training in basic research. Dr. Sakurai had a joint appointment at Columbia and Kyoto University, and put me in touch with his colleague, Dr. Tadashi Isa, a principal investigator in Kyoto and a prominent figure in the Japanese neuroscience community. Dr. Isa agreed to host me over the summer, and I was able to attain funding through two grants, one from Columbia's Global Health Initiative and another through Columbia's Weatherhead East Asian Institute. It is no exaggeration to say that my experience in Dr. Isa's lab was life changing. There I had my first research experience with non-human primates, and I quickly felt that this would soon become my life's work. Macaque monkeys are extremely close to humans in evolutionary history,

with only chimpanzees and bonobos sharing more of our genetic material. For this reason, macaques are in many ways an ideal experimental animal, as they are capable of an extremely wide variety of complex behaviors, cognitive abilities, and emotional processes that are near to our own.

During my time in Dr. Isa's lab, I worked closely with his postdoctoral fellow, Dr. Norihiro Takakuwa, on a project uncovering secondary visual pathways responsible for mediating a phenomenon called blindsight, in which animals with unilateral lesions to V1, the primary visual cortex, were nonetheless able to retain visually-guided saccade behavior when presented with targets in their visual-field defect. I was able to gain experience in a wide variety of tasks related to non-human primate research including eye-tracking, behavioral training, performing muscimol injections, taking electrophysiological recordings, staining and processing histological samples, as well as the care and maintenance of the animals' wellbeing. I also used MATLAB to analyze saccade-performance data we gathered from one experimental animal that had alternatively received muscimol injections to the pulvinar nucleus and the lateral geniculate nucleus of the thalamus. We found that the animal's saccade behavior was impaired somewhat in both of these conditions, with stronger effects seen in the pulvinar inactivation experiments. This tentatively lent evidence for a behaviorally-relevant alternative pathway from the retina to the pulvinar, by way of the superior colliculus. More experiments were necessary to make any definitive claims, but I was able to gain enough hands-on experience to see how such claims could be substantiated if I had been given more time in the lab. Excitingly, these partial results were also supported by findings from my small histological study. There, I processed slices taken from an animal with a long-standing lesion to V1, who had received injections to the superior colliculus with an anterograde labeling adeno-associated virus expressing the fluorescent market dsRed. In that single animal, I was able to identify and describe possible evidence for neuroplastic changes, based on the appearance of labeled axons in the lateral and medial aspects of the pulvinar. Prior literature in control animals had only described projections to the inferior pulvinar, so my findings represented a possible mechanistic explanation for the behavioral findings we observed. At the end of the summer, I presented these histological and behavioral results in an hour-long presentation to members of the lab. Additionally, I presented the project in a poster session for Columbia's Global Health Initiative on my return to New York.

After this extremely positive experience in Dr. Isa's lab, I was certain that I wanted to pursue doctoral training, and that I wanted to do so in a non-human primate lab, if possible. I researched my options at Columbia, which were substantial, and eventually set up a meeting with my current PI, Dr. Daniel Salzman. Dr. Salzman was extremely helpful and encouraging of my plans, and immediately welcomed me into the life of the lab. Furthermore, he encouraged me to increase my chances of receiving doctoral training by applying directly for a position in the PhD program in Neurobiology and Behavior (in addition to applying to transfer into the combined MD/PhD program). After becoming acquainted with Dr. Salzman and the exciting research in his lab, I followed his advice and submitted an application under the assumption that I would join his team to complete my doctoral training. I was accepted into the program and have made the Salzman lab my intellectual home ever since.

Dr. Salzman's longstanding research interests concerned the interactions between the emotional and cognitive systems, specifically with respect to the generation and processing of neural representations of value, or emotional valence. Philosophically, valence is an interesting concept, as certain sense stimuli can exhibit positive valence in one context but negative valence in another context. An everyday example can be seen at a restaurant, where receiving a fork from the waiter might have a high value when accompanied with an entrée, but would have a low value if it had arrived with a bowl of soup. Furthermore, these value representations immediately suggest different behavioral responses – tucking-in versus requesting a spoon, respectively. Prior work in the Salzman lab had shown that the amygdala of non-human primates maintains a representation of this type of context-dependent information, which likely enables behavioral flexibility. When I joined the lab, Dr. Salzman was busy in collaboration with my co-sponsor Dr. Stefano Fusi to publish a manuscript using computational methods to characterize both the content and the geometrical format of neural representations. They had developed a unique reversal-learning paradigm whereby four visual stimuli were assigned different operant responses and different reward outcomes relative to un-cued switches between two contexts. By orthogonalizing the stimulus-response-outcome relationships, they were able to assess whether neural activity from half of the experimental conditions could train a linear decoder to correctly classify context using data from the remaining experimental conditions. When I first learned about this work, I was amazed that animals could perform the task at all (in fact, they perform close to 90% after training), but I was even more intrigued by the idea that the weights of a trained neural network could be used to generate appropriate classifications on data

that the network had never seen before. For this computational feat to work, neural activity in the firing-rate space needed to be of a particular geometry across the various trial types. Since becoming involved in this work, I have gone on to explain this central computational idea to non-technical audiences as a search for 'neural correlates of Platonic forms,' as the structure of the neural activity implies that an idea or concept has been used to make distinctions (i.e. classifications) reliably across different conditions.

However, further investigation into this work made it clear that the structural properties of neural representations had not been tied to successful instances of flexible behavior as animals experienced novel conditions. I decided that my PhD project should further investigate this relationship, and together with Dr. Salzman and Dr. Fusi, I developed a new version of their original task that would allow me to answer this question directly. This project is the subject of the present proposal.

**B. Training Goals and Objectives**

**Overall:** My objectives during the period of the fellowship are aligned towards my career goal of becoming an independent physician-scientist at a leading research institution. Through my training at Columbia University and as a member of the Salzman and Fusi labs, I have already gained substantial scientific, technical, and professional skills. By completing the aims of this proposal, I intend to further the specific proficiencies outlined below. I am excited for the opportunity to target these areas over the course of my NRSA training period, as each will prove to be essential in preparing me for my future career in research.

**In vivo primate electrophysiology:** Since joining Dr. Salzman's lab I have already gained experience recording electrophysiological activity in cortical structures of awake, behaving monkeys. Dr. Salzman's expertise has proved invaluable in acculturating me to the art and science of this important method. Additionally, I have benefitted from direct instruction from several postdoctoral researchers in the lab, most notably Drs. Silvia Bernardi, Roberto Gulli, and David Barack who have each taken personal stock in my development as an electrophysiologist. I will continue to improve my skills in this area, as it is essential for collecting quality data. Through my project, I will have the opportunity to use 24-channel V-probes (Plexon) for simultaneous multi-unit neural recordings, with the possibility of gaining experience with even higher channel-count technologies such as NeuroPixel probes as these become commercially available. Additionally, from exposure to other projects in our lab and the neighboring lab of Dr. Yasmine El-Shamayleh, I will have the opportunity to gain exposure to the increasingly important causal manipulations being used in primate electrophysiology, such as cortical microstimulation as well as chemogenetic and optogenetic approaches.

**Data analysis and visualization:** The conceptual advances proposed by this project hinge on sophisticated machine-learning methods, with which I have already gained significant familiarity. Nonetheless, I will continue learning about these topics independently and through targeted readings and discussions with Drs. Salzman and Fusi. in order to improve the methods and increase the clarity of my data summaries. In addition to more modern techniques, I will of course be applying standard statistical tests in summarizing both behavioral and neural data, under the supervision of my mentors and the many competent postdoctoral researchers across the two labs.

**Theoretical approaches to neuroscience:** I was motivated to seek co-mentorship from Dr. Fusi because of his well-known contributions to the field of theoretical neuroscience and his longstanding collaboration with my sponsor, Dr. Salzman. While my ambition is not to become a theoretician, my goal is to gain enough fluency in the methods and current debates to have high-level collaborations with theoretical neuroscientists. In spite of my lack of formal training prior to graduate school, through self-study and an introductory course in neural data analysis, I prepared myself to enroll in Columbia's introductory course in theoretical neuroscience. I completed this course in spring of 2020, and in future years I plan to continue this formal training through the advanced theory seminar offered in the department. In addition, I receive informal training in theory by attending both in-house and guest lab meetings held by the Theory Center each week. Finally, I will use my thesis project to deepen my understanding of these topics with hands-on experience, under the direct guidance of Dr. Fusi and through collaborations with his lab members.

**Primate behavioral training:** I have gained much experience and confidence until now training non-human primates, but I recognize that I am still relatively new to this work. All primate systems neuroscience hinges on the experimenter's ability to train the animals on the task in question. Therefore, I will continue to work with our

lab's primate technician, the relevant postdoctoral fellows, as well as the veterinary staff and behavioral specialists employed by the institution to hone these skills as much as possible during the behavioral training period of my project and beyond.

**Surgical skills:** I am eager to develop my surgical skills as much as possible during my time in the lab. So far, I have served as assistant surgeon on several routine procedures, such as implant removals and dural scrapes. I plan to work with the expert surgeons in the lab and in neighboring labs to gain competence and confidence in a variety of procedures, with the goal of being able to regularly serve as lead surgeon by the time I finish my training. These skills will help make me an attractive candidate for any future primate labs I should like to join.

**Engineering skills for neuroscience research:** Knowing what tools to use is essential for properly setting up an experiment. Often times, however, the best tool is one that does not yet exist. I have had some experience with this since joining the lab, and have enjoyed working with all three of the permanent staff engineers in the Advanced Instrumentation Core to develop custom equipment ranging from integrated circuits, to optical sensors (with the help of Dr. Lee Lovejoy in our lab), to structural supports and animal positioning apparatuses for my experimental rig. As the need arises in the course of my work for engineering solutions, I look forward to being able to work further with the Advanced Instrumentation engineers to develop the best tools for the job.

**Scientific writing and grantsmanship**: I aim to strengthen my writing skills for scientific manuscripts and grants because these skills are essential for my future success as a principal investigator. The department of Neurobiology and Behavior provides excellent, required courses on writing fellowship applications and journal articles, where I will be able to get targeted feedback from both classmates and faculty. Additionally, I will work with Dr. Salzman to polish and refine all written work to be submitted for publication, as clarity and style are essential in communicating abstract scientific concepts and conclusions. A major objective mine is to publish a manuscript in a high-impact journal as the culmination of my doctoral research. I expect to be able to publish results from this project by 2023. In the meantime, to supplement my publication record, I am drafting a review article with Dr. Silvia Bernardi on abstraction and psychiatric conditions, combining my basic science research with my clinical interests and experience. We plan to submit this manuscript for publication by summer 2021. Although do not have extensive experience in scientific writing, I am confident that my humanities background, along with the expert guidance of my mentor, will allow me to refine these skills and successfully navigate the publication process. My co-mentor, who is also well-published, will provide additional input, specifically with respect to descriptions of models and theoretical considerations. As with the writing of this fellowship and previous grants, I will also receive feedback from postdoctoral fellows in the lab. I am therefore confident that I will receive the support and training I need to achieve these goals.

**Oral presentation:** Presentation skills are absolutely essential for a successful career in science, and I will use multiple opportunities to practice and improve in this arena. During lab meetings, I have already received a great deal of constructive criticism regarding my presentation style, and I will continue to seek this feedback as I present partial results over the course of my project. As in the area of written communication, Dr. Salzman has developed and impeccable communication style, and I take his opinion very seriously when he gives me suggestions. Thankfully, from my background in performing arts, I do not suffer from stage fright, however I know that I have much to learn in terms of keeping scientific audiences engaged. I will have many opportunities to practice this skill, such as in the monthly Research in Progress seminars, which is a required speaking engagement for all graduate students in the program to present their research to an audience of students, postdocs and faculty in the department. Lastly, I will seek out opportunities to give talks at national and international conferences as my project unfolds. In particular, I plan to attend future Cosyne, Cognitive Computational Neuroscience, and Gordon conferences, amongst others. In preparation for talks like these, Dr. Salzman feels strongly about providing a forum for practice talks, where he and other members of the lab can give feedback in a respectful and constructive way. I have participated already in many such practice talks for other members of the lab, and I can see the immense value that these opportunities will provide.

**Teaching and Career development:** Teaching both gives me great pleasure and allows me to share my passion for neuroscience with interested students and potential future colleagues. In addition to my experience teaching in Japan, I had the opportunity to TA for Columbia's undergraduate introductory course in neurobiology in fall 2019. I plan to continue such work for at least one semester per year for the remainder of my graduate training. Aside from teaching, Columbia offers many opportunities for scientific career development. Students take

professional skills workshops run by Dr. Carol Mason that provide training in searching for scientific jobs, networking within the scientific community, and navigating the peer review process. Also, the Zuckerman Institute and Center for Theoretical Neuroscience each offer weekly seminars, which provide regular opportunities for intellectual development and networking. I will take advantage of these opportunities, in addition to continuing my regular weekly discussions with Dr. Salzman about my career path, as I work to achieve my goals.

### C. Activities Planned Under This Award

By the time this grant comes into effect, I will be focusing the majority of my time on the completion of my specific aims. At the time of writing, I have finished all my required coursework but will pursue elective coursework to further develop my quantitative skills and to complement my independent reading. I will also serve as a teaching assistant one semester per year, in the graduate-level course Data Analysis for Neuroscientists or in the undergraduate-level Introduction to Systems Neuroscience, as teaching allows me both to deepen my own competencies and to contribute to the academic community. Additionally, I will continue participating in the weekly lab-wide journal club, data meeting, and abstraction working-group meetings. I will formally present at least once per semester in each of these settings. I will also continue to improve my communication and presentation skills by presenting for my thesis committee every 6-9 months. To deepen my exposure to a wide range of topics in neuroscience, I will also attend the weekly seminars hosted by the Neurobiology Department and the Theory Center.

As outlined above, I anticipate being able to submit a review article for publication by the end of summer 2021, with results to be published by 2023.

I will allocate my time during the course of the fellowship according to the table below. I will plan to accomplish Aim 1 (behavioral training) over the course of the first two funding years. I will then start collecting neural data and begin analyses of the behavioral data. All data collection will finish by the end the third funding year. The remaining two years of the grant will be spent finalizing the data analysis and writing my thesis manuscript.

| Funding Year | Aim 1 | Aim 2 | Writing: Grants, Papers, Thesis | Meetings/ Presentations | Teaching |
|---|---|---|---|---|---|
| 1 | 60% | 10% | 10% | 10% | 10% |
| 2 | 50% | 20% | 10% | 10% | 10% |
| 3 | 20% | 50% | 10% | 10% | 10% |

**SPECIFIC AIMS**

Pursuing goals in a complex environment requires behavioral flexibility – the ability to rapidly adjust actions and expectations in response to changes in internal and environmental variables. Deficits in flexible behavior are seen in nearly all psychiatric conditions, including addiction, anxiety, mood, and psychotic disorders. However, the neural basis of behavioral flexibility remains poorly understood. This proposal will study two important types of flexibility: **Type 1**, rapid context-dependent adjustments in behavior, and **Type 2**, rapid decision-making in novel conditions through generalization from past experiences. These types of flexibility make distinct demands on neural representations [1–4]. The dorsolateral prefrontal cortex (DLPFC) [5–9] and hippocampus (HPC) [1, 10–14] are thought to contribute to both types of flexibility. This proposal seeks to delineate each area's distinct computational role in these two types of flexible behavior.

Recent work has suggested that analyzing the *geometry* of neural representations can illuminate the computational role of a neural ensemble in supporting both types of flexibility [15, 16]. Geometry here refers to the structured relationships among firing patterns in a neural ensemble that are elicited by different conditions. In the context of experimental data, these relationships can be visualized by plotting the firing rate of $n$ neurons for each of $m$ experimental conditions $\{s_1, s_2, \ldots, s_m\}$. The $n$-dimensions of the plot correspond to the firing-rate of each neuron, and each of $m$ points corresponds to the firing pattern elicited by an experimental condition. Each experimental condition is defined by the values of the task variables. Recent work suggests that **Type 1** flexibility requires a geometry in which a linear decoder can read out the value of as many variables as possible. This is because different variables acquire behavioral significance depending on task demands, which are often context-dependent. Flexible context-dependent adjustments in behavior will fail if too few variables are represented. **Type 2** flexibility demands a geometry that has certain generalization properties. Specifically, as will be explained below, to support generalization in a novel condition, the geometry should allow a linear decoder to read out variables in novel conditions *in the same way* it reads out variables for familiar conditions. Two measures of the geometry of representations have been proposed to support these types of flexibility [15]. The first, shattering-dimensionality (SD), quantifies how many variables can be read out from a neural representation. The second, cross-condition generalization performance (CCGP), quantifies how well a linear decoder trained to read out a variable from *some* conditions can accurately read out the same variable from conditions not used for training. HPC and DLPFC both contain representations that simultaneously exhibit high SD and high CCGP [15]. However, a relationship between these measures and these two types of flexible behavior remains to be established. I propose to **investigate the behavioral relevance of representational geometry** in HPC and DLPFC as monkeys perform a task requiring both types of behavioral flexibility on separate trials. This proposal will test the **hypothesis** that Type 1 flexibility correlates with SD, and Type 2 flexibility correlates with CCGP.

**Aim 1: To determine if and where shattering-dimensionality (SD) correlates with context-dependent behavioral flexibility.** Monkeys will perform a serial reversal-learning task in which they switch between two contexts, each defined by four experimental conditions. Each experimental condition corresponds to a unique, context-dependent stimulus-response-outcome (SRO) mapping for one of the four visual stimuli used. On the first 8 trials of each block, contextual cues will appear after presentation of the visual stimulus and a brief delay period. Animals will perform many trials in one context before being presented with a switch cue, signaling 50% probability of a context switch on the next trial. Neural responses will be analyzed in the following trial during the delay period that precedes the contextual cue. Since context is uncertain during this period, animals will need to represent all 8 SRO mappings in order to select the correct response after being informed of the context, an example of Type 1 flexibility. As these trials rely on working memory and task switching for the 8 SRO mappings, I hypothesize that the correlation between SD and behavior will be strongest in DLPFC as compared to HPC.

**Aim 2: To determine if and where cross-condition generalization performance (CCGP) correlates with successful generalization in novel conditions.** After performing several blocks of the task in Aim 1, two new contextual cues will be introduced for the two behavioral contexts (again, on the first 8 trials of each block). However, now animals will only be exposed to conditions using three of the four visual stimuli. After experiencing many trials in both contexts, the fourth stimulus will appear for the first time on a trial following a switch cue. Because animals have never experienced the new contextual cues with this stimulus, such trials function as novel conditions. Monkeys can exhibit generalization on this trial by remembering from prior experience that the fourth stimulus is linked to the other three within a context and acting appropriately, an example of Type 2 flexibility. As in Aim 1, neural responses will be analyzed during the delay period following stimulus presentation. Because of the HPCs role in pattern completion and contextual associations and the DLPFCs role in working memory and rule-related processing, I hypothesize that performance on these trials will correlate with CCGP in both HPC and DLPFC.

## RESEARCH STRATEGY

### 1. SIGNIFICANCE:

*Overview:* We live in a world that is constantly changing. In order to pursue goals and survive in such a complex environment, one cannot rely on learned stimulus-response associations alone. Rather, we require behavioral flexibility – the ability to rapidly adjust actions and expectations in response to changes in internal and environmental variables. Our ability to engage in flexible behavior breaks down under conditions of stress and in the setting of many neuropsychiatric conditions, including addiction, anxiety, mood, and psychotic disorders [17–21]. This project aims to characterize the underlying neurophysiology that gives rise to two important types of behavioral flexibility: **Type 1**, rapid context-dependent adjustments in behavior, and **Type 2**, rapid decision-making in novel conditions through generalization from past experiences. These types of flexibility make distinct demands on neural representations [1–4]. The dorsolateral prefrontal cortex (DLPFC) [5–9] and hippocampus (HPC) [1, 10–14] are thought to contribute to both types of flexibility, but delineating each area's unique computational role has proved difficult. The overarching hypothesis of this grant is that neural representations of variables – considered as the pattern of activity in a population across experimental conditions - must exhibit a particular *geometry* to support the two types of behavioral flexibility. I will test this hypothesis by comparing the geometry of representations during correct and incorrect behavior on certain trial types. By elucidating the neural basis of flexibility in HPC and DLPFC, this project provides a foundation for understanding the neural basis of deficits seen in stress and neuropsychiatric illness, paving the way for the development of future treatments.

*Neural Representations in Systems Neuroscience:* Neural representations can be considered to be the patterns of activity in across a neural ensemble observed in relation to modulations in internal and environmental variables. Neurons can represent variables that describe "explicit" features of the world (e.g. physical properties or internal states like thirst), and "hidden" (or latent) features, such as relationships between objects or other features [22]. Traditionally, research in neuroscience has sought to measure the *content* of neural representations in efforts to explain complex behaviors. In this way, researchers have identified many important variables that are involved in computations like object recognition, category formation, and rule-based decision-making [23–26]. Efforts of this kind typically begin by postulating that some variable is necessary for the behavior in question and proceed by searching for representations of that variable in candidate brain areas, during key task epochs. However, such studies have typically not distinguished between different types of flexible behavior and how these types place distinct demands in how a neural ensemble represents specific variables to support behavioral flexibility.

*The Geometry of Neural Representations:* In contrast to this traditional approach, recent work has suggested that analyzing the *geometry* of neural representations can illuminate the computational role of a neural ensemble in supporting Type 1 and Type 2 flexibility [15, 16]. Geometry here refers to the structured relationships among firing patterns in a neural ensemble that are elicited by different conditions. In the context of experimental data, these relationships can be visualized by plotting the firing rate of $n$ neurons for each of $m$ experimental conditions $\{s_1, s_2, \ldots, s_m\}$. The $n$ -dimensions of the plot correspond to the firing-rate of each neuron, and each of the $m$ points correspond to the firing pattern elicited by an experimental condition. Each experimental condition is defined by the values of

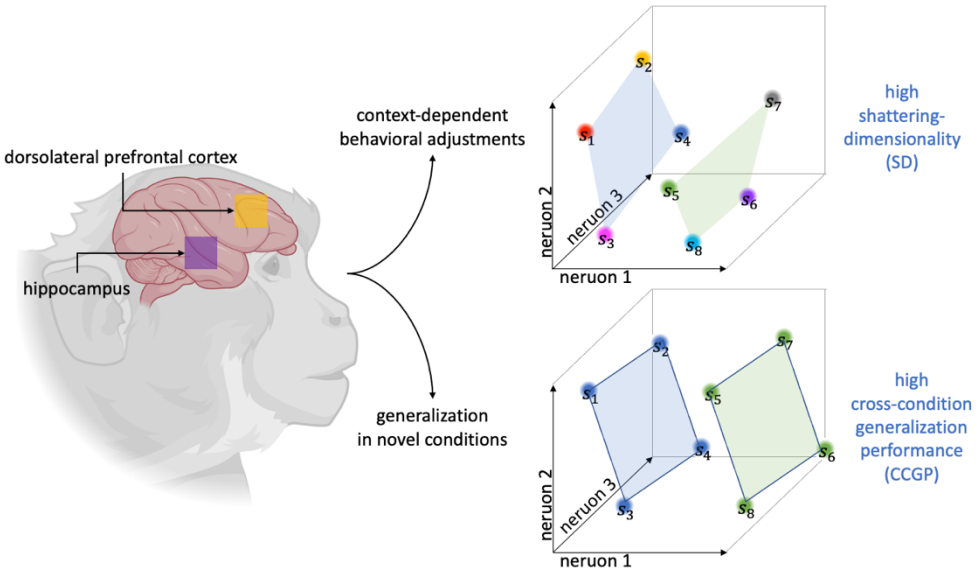


**Fig. 1: Behavioral flexibility and the geometry of neural representations.** (left) Targeted brain areas in the rhesus macaque. (right) Schematic showing predicted geometries for the two types of behavioral flexibility. Plots show the response of three neurons to 8 experimental conditions belonging to two contexts (blue & green). The top shows a geometry in which all conditions (and variables) are highly separable by a linear decoder, as the representation has high SD. The bottom shows a geometry in which conditions are highly structured across the two contexts, yielding high CCGP and somewhat lower SD.

the task variables, such as stimulus identity, operant response, and reward contingencies. The plots in Fig. 1 schematize two different geometries for 8 experimental conditions across three neurons. Based on each neuron's selectivity for the variables that comprise each condition, the 8 experimental conditions appear as points in specific regions of the firing-rate space. The center of each point represents the average activity across many trials under that experimental condition. In this figure, the responses of 3 neurons are plotted against each other for each representation, but the intuition extends to higher neuron counts as well.

Recent research from the Salzman and Fusi labs has suggested that **Type 1** flexibility requires a geometry in which a linear decoder can read out the value of as many variables as possible. This is because different variables acquire behavioral significance depending on task demands, which are often context-dependent. Flexible context-dependent adjustments in behavior will fail if too few variables are represented, as the proper input-output mappings cannot be generated if the relevant variables that define such mappings are not present in neural activity. Geometry of this kind is schematized in the upper plot in Fig. 1, where each experimental condition randomly occupies a separate region of the firing-rate space. In fact, because of the arrangement of the points, assuming noise is not too high, a linear decoder can separate all possible groupings of 4 conditions from the remaining 4 conditions. Thus any variable can be read out in relation to task demands.

In contrast, **Type 2** flexibility demands a geometry that has certain generalization properties. Specifically, to support generalization in a novel condition, the geometry should allow a linear decoder to read out variables in novel conditions *in the same way* it reads out variables for familiar conditions. This can occur because the world is structured, and novel conditions often share features with familiar conditions. If the brain represents such structured relationships between conditions, it facilitates generalization, as neural responses to novel conditions can use the brain's pre-existing encoding scheme for the novel condition's constituent variables. Such a geometry is shown in the lower plot of Fig. 1, where experimental conditions are not only organized by context, but also along axes of symmetry that could correspond to additional task variables.

*Two Measures of Geometry:* The Salzman and Fusi labs have developed two tools to measure the geometric properties thought to underlie Type 1 and Type 2 flexibility [15]. The first, called **shattering-dimensionality (SD)**, quantifies how many variables a linear decoder can be read out from a neural representation. This is schematized in Fig. 2, which shows an example representation of four experimental conditions across two neurons. Together, the 7 panels of the figure show all possible binary variables that could be defined across these 4 conditions. Within each panel, each experimental condition is assigned an arbitrary color, corresponding to the value of the binary variable in question. For example, in Fig. 2a, condition $s_1$ is red, while the other conditions are yellow, indicating that the variable in question takes one value for $s_1$ and another value for the remaining conditions. Using linear decoders, a boundary can be generated that optimally segregates conditions with respect to the variable in question. These decoders are trained with data from *all* experimental conditions that differ with respect to this variable (in contrast to the second method, described below). This boundary is shown as a dotted line in Figs. 2 a-f. By contrast, Fig. 2g shows a variable assignment for which no such boundary can be generated. Because linear decoders can successfully read out variables from 6 of the 7 possible assignments, this representation has a SD of 6/7 = 0.86.

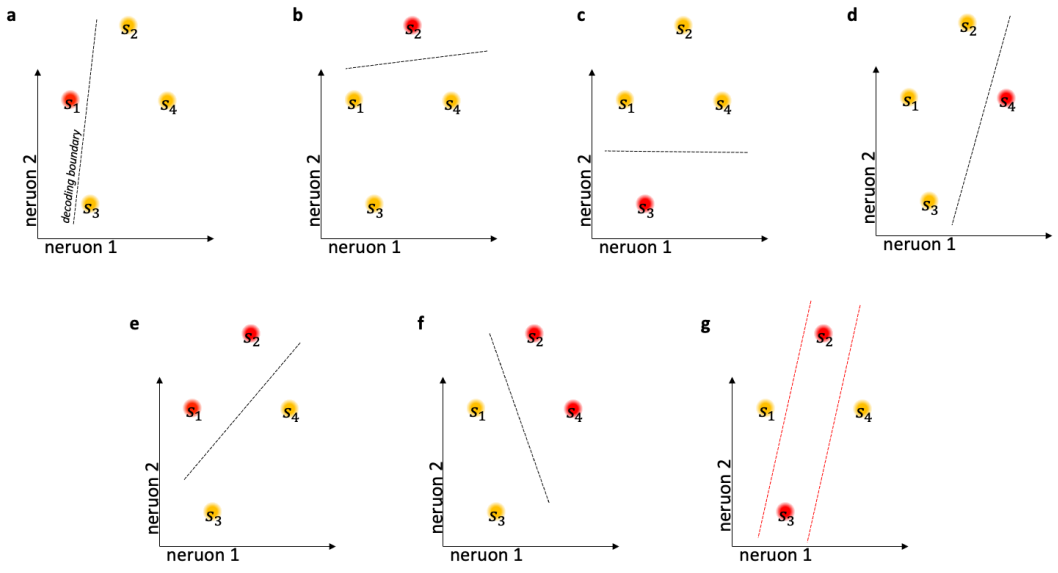


**Fig. 2: Computing shattering-dimensionality (SD).** Example representation for four experimental conditions across two neurons. Each panel shows one of the 7 arbitrary variables that could be read out from these four experimental conditions (visualized as yellow vs. red). The dotted line in panels (a-f) show how a linear decoder could separate the four conditions according to the variable in question when training on a subset of *all* conditions. Panel (g) shows a geometry that does not yield linear separability using a decoder of this kind. Therefore, SD for this representation would be 6/7 = 0.86.

Again, this example generalizes to higher-dimensional representations with more neurons and experimental conditions.

The second measure of geometry, called **cross-condition generalization performance (CCGP)**, quantifies how well a linear decoder trained to read out a variable from *some* conditions can accurately read out the same variable from conditions not used for training. This is shown in Fig. 3, which again plots the activity of two neurons against each other for four experimental conditions, where two binary variables describe each condition. To calculate CCGP, linear decoders are again trained to segregate conditions, but this time only using data from *some* conditions, called the training set. This again will yield a boundary that maximally separates conditions based on the variable in question. Then, using this pre-trained network, we evaluate how well this boundary can be used to correctly classify conditions in the testing set according to the variable in question. This is performed for every possible assignment of training and testing sets. A boundary line is shown, which is derived from training the decoder on the designated subset of experimental conditions. Performance of this trained decoder to read out the value of the variable on the testing set varies depending upon the training set. CCGP is the average performance across all possible training/testing sets. For Fig. 3a, CCGP is therefore (1 + 1 + 0.5 + 0.5) ÷ 4 = 0.75. By contrast, if a variable is represented by the pattern of activity shown in Fig. 3b, the geometry does not produce high CCGP. Here CCGP is 0 for the depicted variable. CCGP can be calculated for any and all possible groupings of experimental conditions, where each grouping can be considered a variable.

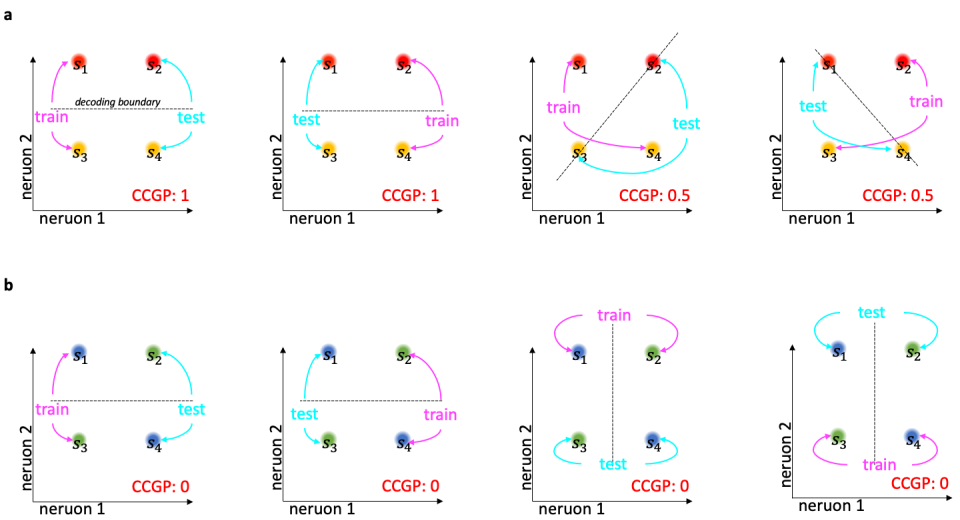


**Fig. 3: Computing cross-condition generalization performance (CCGP).** Two examples showing a representation as in Fig. 2, with four experimental conditions across two neurons. Panel (a) shows one variable assignment (a red/ yellow dichotomy), while panel (b) shows an alternative variable assignment (blue/ green). The dotted line in each panel shows how a linear decoder could separate the two training conditions. We then measure how well how this decoding boundary generalizes to correctly classify the testing conditions. The variable in panel (a) shows successful generalization properties in the first two sub-panels, while it generalizes at chance levels for the second two. The average CCGP for this variable representation is therefore 0.75. By contrast, panel (b) shows no such generalization properties, with an average CCGP of 0.

Of note, both SD and CCGP differ from traditional investigations of neural representations, as 1) these analyses measure the geometry of representations, and 2) these measures not only characterize 'task-relevant' variables but all possible variables that can be assigned to the representation. The methods thereby describe the geometry of representations in a completely unbiased manner [15].

*Prior Results and Outstanding Questions:* Previous work in the lab has examined the geometry of representations using data from a serial reversal-learning task and demonstrated that HPC and DLPFC both contain representations that simultaneously exhibit high SD and high CCGP [15]. Importantly, this work confirmed theoretical predictions that variables may not exhibit high CCGP, even when they are decodable using traditional methods.

However, a relationship between these measures and these two types of flexible behavior remains to be established, as previous studies did not require animals to generalize in novel conditions. This project stands to make crucial advancements over our earlier work by introducing a task that requires both types of behavioral flexibility on separate trials. Together with these theoretical tools, this project promises to be the first to demonstrate the role of the geometry of neural representations in behavioral flexibility, potentially revealing a new and important population-level neural coding scheme [3, 27–29]. My **hypothesis** that Type 1 flexibility correlates with SD, and Type 2 flexibility correlates with CCGP in these brain areas is summarized in Fig. 1.

*Potential Impact:* This project will contribute to the field in three important ways. 1) This will be the first study to show specific evidence connecting the geometry of neural representations to the performance of Type 1 and Type 2 behavioral flexibility. Focusing on the *geometry* of representations contrasts with dominant approaches in neuroscience that have only examined the *content* of variables being represented. While my hypothesis posits

that these types of behavioral flexibility are inherently dependent on the geometry of neural representations, these are by no means the only behaviors for which geometry might play an essential role. Demonstrating the importance of geometry of representations for complex behavior will open the field to further investigations into coding schemes of this kind. 2) This study represents a unique neurobiological application of the recent advances in machine-learning and theories of neural computation. In machine-learning, generalization is assessed by testing a network's classification performance using samples that are different in some important respect from the training samples. By employing a symmetric task-design with novel stimuli, I will be able to apply this definition to the analysis of my neural data, training a network on *some* experimental conditions and testing its classification performance on *other* experimental conditions. By correlating this cross-condition generalization performance with behavioral generalization, this project has the potential to reveal an important computational mechanism behind the generation of flexible behavior in novel situations. 3) By performing high channel-count recordings from two different brain areas simultaneously, I will be able to compare the time-dependent correlations between geometric format and behavioral performance in these areas. This will provide a new approach to delineate the function of different brain areas, paving the way for development of future treatments for neuropsychiatric illness.

## 2. APPROACH:

*Overall Methods:* Animals will be trained to perform a task that requires Type 1 and Type 2 behavioral flexibility on separate trials. During behavior, I will perform simultaneous electrophysiological recordings of HPC and DLPFC. HPC will include recordings from across CA1, CA2, CA3, and DG. DLPFC recordings will be taken from Broadman areas 8, 9, and 46. Recordings will be made continuously using 1-2 32-channel vertical electrodes (V-Probes, Plexon) in each brain area. Analog signals will be amplified, filtered, and digitized, after which, each channel will undergo automated spike sorting using MountainSort [30] to separate single units within each channel, which will additionally be verified manually using the Plexon offline sorter platform. Measures of geometry will be performed according to the procedures outlined in the Aims below.

*Sex as a Biological Variable:* In primate electrophysiology studies, studies are typically made in 2 experimental subjects, making it impossible to study sex as a biological variable. Moreover, male monkeys are typically employed because they are more readily available for purchase, with females often reserved for use in breeding colonies. As such, we currently have no female animals in our lab. Because of these limitations, meaningful comparisons of findings across gender will not be possible in the present study.

**Aim 1: To determine if and where shattering-dimensionality (SD) correlates with context-dependent behavioral flexibility.**

*Rationale:* Context-dependent behavior requires keeping track of many variables that describe features of each context. Context-depending behavior should suffer when too few variables are represented. In this Aim, monkeys will perform a task that requires context-dependent behavioral flexibility. I will analyze the shattering-dimensionality (SD) of neural representations in successful and unsuccessful trials to assess how geometries that encode many variables can support this type of flexibility.

*Experimental Procedures:* Two male rhesus macaque monkeys will be trained to perform a serial reversal-learning task in which they switch between two contexts, each defined by four experimental conditions. Each experimental condition corresponds to a unique, context-dependent stimulus-response-outcome (SRO) mapping for one of the four visual stimuli used. The visual stimuli will be computer-generated fractal images (A-D), the operant responses will be one of four saccadic eye movements (up, down, left, right), and the outcome of successful trials will be either a liquid reward or no reward. Each of these SRO mappings is shown in Fig. 4a. This design ensures that any given operant response is not preferentially linked to a particular reinforcement outcome, though fractals B and D remain rewarded and unrewarded across both contexts, respectively. Fig. 4b shows the trial structure. Animals maintain central fixation for 500 ms, followed by stimulus presentation for 300 ms. On the first 8 trials of each block, contextual cues (also fractal images) will appear for 300 ms following a delay period of 1000 ms. For the subsequent trials in a block, no contextual cue will appear. Saccade targets will appear during a second delay period of 1000 ms, and the removal of the central fixation point will signal the animal to respond. Correct responses will be followed by a liquid reward, depending on the SRO mapping for the experimental condition.

Animals will perform many trials in one context before being presented with a switch cue, signaling 50% probability of a context switch on the next trial. In the following trial, context will be maximally uncertain, and

animals will need to wait for the contextual cue to know the correct response and outcome. Thus, the task demands that the monkeys represent all possible 8 experimental conditions (SRO mappings) in a distinct manner pending being informed of the context. Only then can the monkey determine the correct response by knowing the stimulus and the context and the associated contingencies. On trials in which context does not change, monkeys will be presented with another switch-cue trial until they enter a new context. This block structure is shown in Fig. 4c. To keep animals engaged, the base set of visual stimuli (A-D) will be replaced after several days of consistently high performance. While animals perform the task, several behavioral measures will be recorded, including anticipatory licking behavior, and continuous eye-movement tracking and pupillometry. Anticipatory licking will be recorded with a custom-built apparatus. Eye movements and pupillometry will be recorded with commercial equipment from SR Systems (EyeLink).

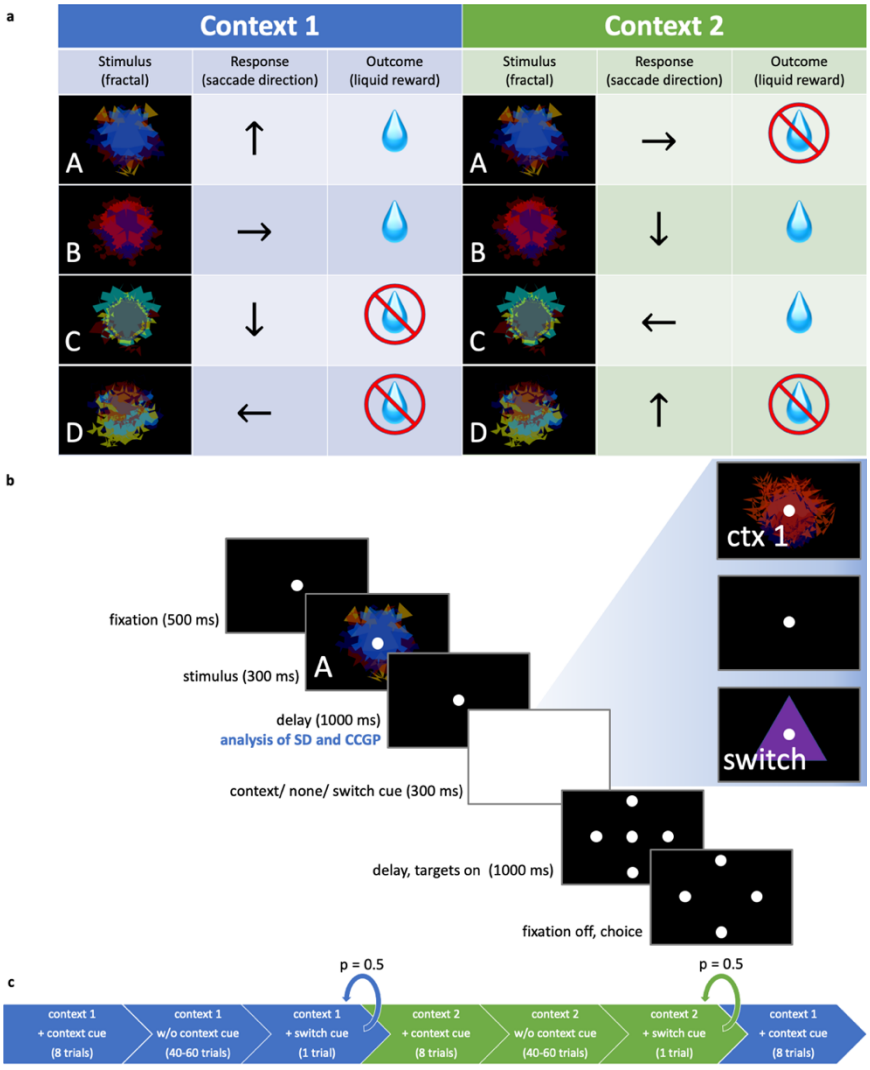


**Fig. 4: Overall task design.** (a) 8 experimental conditions, defined by four unique stimulus-response-outcome (SRO) mappings across two contexts. (b) Trial structure. Neural activity will be analyzed in the first delay period, following stimulus presentation. The inset shows cues that could appear in context 1 following the first delay period: a contextual cue on the first 8 trials in a block, no cue on subsequent trials, or a switch cue at the end of a block. (c) Block structure, showing transitions between contexts.

*Preliminary Studies:* This task combines features of tasks previously used in successful studies with behaving monkeys. Namely, it adapts the reversal-learning task in [15], by introducing saccadic eye movements as the operant response, analogous to the design of [31]. However, the contextual and switch cues are novel features of this task design, which serve to create a trace interval in which to analyze the representational geometry preceding behavior. Training has already begun with the first monkey, who is showing evidence of contextual switching using only two of the four fractal images (data not shown).

*Expected Outcomes:* Neural responses will be primarily analyzed in trials following a switch-cue trial, during the delay period that precedes the contextual cue. Since context is uncertain during this period, animals will need to represent all 8 SRO mappings in order to select the correct response after being informed of the context, an example of Type 1 flexibility. I will compute SD separately using data from successful and unsuccessful trials to correlate this measure of geometry with performance. As these trials rely on working memory and task switching, known functions of DLPFC, I hypothesize that the correlation between SD and behavior will be strongest in DLPFC as compared to HPC.

*Potential Pitfalls:* As with all animal studies, the possibility of multiple behavioral tactics presents the greatest potential pitfall. Namely, there is some concern that animals could select an action on seeing the stimulus, and then switch the action if they are informed of a change in context. However, this tactic is highly unlikely in the current task design, as each stimulus is associated with two different target directions. If the task had used a binary operant response as in [15], this tactic would have posed a greater concern.

Another possible pitfall could arise if animals perform too well on the task, thereby decreasing the number of error trials available for analysis. Should this situation occur, it can be remedied through small changes in the

task parameters. For instance, animals can be introduced to a new base set of visual stimuli more frequently to increase the difficulty of the task.

**Aim 2: To determine if and where cross-condition generalization performance (CCGP) correlates with successful generalization in novel conditions.**

*Rationale:* Generalization from past conditions to novel ones requires that the brain represent one or more variables in a manner that can readily classify the novel condition. This can occur if the geometry of a representation already reflects the links between variables comprising familiar (already experienced) and novel conditions. Here I will test whether such geometries correlate with Type 2 flexibility, by comparing the cross-condition generalization performance (CCGP) of neural representations in successful and unsuccessful instances of generalization in novel conditions.

*Experimental Procedures:* This aim adapts the task in Aim 1, but introduces many trials in which animals must generalize in novel conditions. The block structure for this task shown in Fig. 5. After performing several blocks of the task in Aim 1, two new contextual cues (fractal images) will be introduced for the two behavioral contexts (again, on the first 8 trials of each block). However, now animals will only be exposed to conditions using three of the four visual stimuli. After experiencing many trials in both contexts, the fourth stimulus will appear for the first time on a trial following a switch cue. Because animals have never experienced the new contextual cues with this stimulus, such trials function as novel conditions. Monkeys can exhibit generalization on this trial by remembering from prior experience that the fourth stimulus is linked to the other three within a context and acting appropriately, an example of Type 2 flexibility.

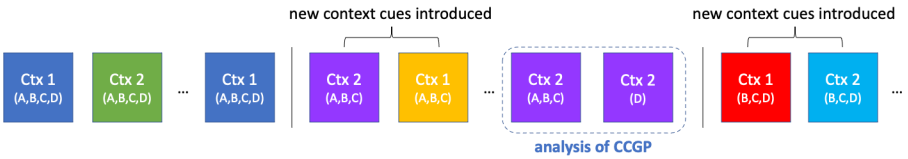


**Fig. 5: Task design for Aim 2.** At first, animals will experience several blocks in each context with all four visual stimuli (A-D). Then new contextual cues will be introduced, and animals will only experience three of the four visual stimuli (A-C, in the first example), before experiencing the held-out stimulus for the first time on a trial following a switch-cue trial. Importantly, as in Aim 1, switch-cue trials will precede all changes in context, with the switch cue signaling a 50% chance of changing context in the following trial. The example here shows the held-out stimulus (D) appearing on a trial when context remains the same.

*Expected Outcomes:* As in Aim 1, neural responses will be primarily analyzed during the delay period following stimulus presentation. I will compute CCGP separately for the variable context using data from successful and unsuccessful trials to correlate this measure of geometry with Type 2 flexibility. To do so, I will analyze activity from the delay period following switch-cue trials, which will include data from the three previously experienced conditions as well as the held-out (novel) condition, as indicated by the dotted line in the figure. Across both contexts, this will yield delay period activity from all 8 experimental conditions. The HPC has long been implicated in the formation of episodic memories [32–34], which due to their relational nature, might be involved in the creation and maintenance of representations supporting generalization. Human studies suggest that the HPC is involved in conceptual knowledge and learning of statistical regularities across events [35, 36]. In single-neuron experiments, DLPFC has also been shown to encode rule and category-based information in working memory [24, 25, 37–39]. Furthermore, both of these areas have been implicated in representing variables related to both novelty and task structure [26, 40–42]. Against this background, I hypothesize that performance on these trials will correlate with CCGP in both HPC and DLPFC. Note that this version of the task, as in Aim 1, employs a switch cue signaling a 50% chance of changing context on the next trial. Consequently, since the context in effect is not indicated until the end of the delay period on the following trial, there is maximal uncertainty as to which SRO mapping might be needed. SD should therefore remain high to support task performance here as well. This implies that neural ensembles may realize a geometry with *both* high CCGP for context and high SD, as was observed in [15]. I therefore predict that performance on trials following a switch cue should also be correlated with SD, as monkeys must link the held-out stimulus to the correct context for generalization *and* must represent the 8 SRO mappings for context-dependent flexibility.

*Potential Pitfalls:* One potential pitfall is that I will fail to see the predicted trends in failure trials due to changes in geometry across the learning process (i.e., as animals *learn to generalize*). It is conceivable that earlier trials are marked by lower CCGP scores in both correct and error trials, which might diminish any comparisons made between the two outcomes. Two approaches are possible in mitigating this confound. First, I will conduct separate analyses by learning epoch, to show that CCGP score increases with overall performance on correct trials but fails to do so with error trials. Also, I will restrict my analysis to trials made only after a criterion level of performance is reached, thereby controlling for the learning process.

## RESPECTIVE CONTRIBUTIONS

This project will provide insight into the relationship between two types of behavioral flexibility and the geometry of neural representations in the hippocampus (HPC) and dorsolateral prefrontal cortex (DLPFC). The first type of behavioral flexibility facilitates switching behavior between contexts, and the second type facilitates generalizing in novel conditions. This proposal was developed in collaboration between myself, my advisor, Dr. Daniel Salzman, and my co-advisor, Dr. Stefano Fusi. It is a direct extension of prior collaborative work between my advisors, which sought to characterize the geometry of neural representations in the HPC and DLPFC. The task proposed in this project grew out of discussions concerning the limitations of that original study, which did not require animals to respond to novel experimental conditions. I drafted the research proposal and improved upon it based on feedback from Dr. Salzman. I will execute all experiments described in the proposal, and I will also be responsible for training the monkeys on the task each day. I will lead or assist with all surgeries, under the supervision of the veterinary staff and senior surgeons in the Salzman lab. Data analysis will be conducted by myself, under the supervision of Dr. Salzman and Dr. Fusi. Dr. Salzman and I jointly developed the research training plan, which is tailored toward my specific career goal of becoming an independent physician scientist studying the mechanisms behind emotional and cognitive processing in the brain.

## SELECTION OF SPONSOR AND INSTITUTION

**Dr. Daniel Salzman:** My decision to pursue graduate work in Columbia's Neurobiology and Behavior program went hand in hand with my decision to join Dr. Daniel Salzman's lab. When I first met Dr. Salzman, I was a student at Columbia's Vagelos College of Physicians & Surgeons, beginning the second year of my MD degree. In my first semester of medical school, I had started taking definitive steps toward pursuing formal training in research. It occurred to me that the most obvious and rigorous way to do so would be to transfer into Columbia's combined MD /PhD program. When I explored this option with one of the program directors, she warned me that it was unlikely that I would be able to transfer, but that my chances at doing so might be increased if I could find a suitable lab and a willing mentor. Over the next two semesters I was able to organize two short "rotations" shadowing graduate students in my spare time from medical school. Following these experiences, I had gained exposure to neuroscience research at the molecular, cellular, disease-model, and local circuit-levels, but had yet to explore more integrative, systems-level questions about the generation of behavior. I had the opportunity to do so in the summer following my first year of medical school, when I traveled to Japan to rotate in Dr. Tadashi Isa's laboratory at Kyoto University. This was my first experience working with non-human primates, and I found the research to be extremely exciting. I read about the incredible history of systems neuroscience research using macaque monkeys, and I saw clearly how these animals could provide unique access to the mechanistic study of thoughts, feelings, and complex behaviors. When I returned from Japan, I started to research the many primate labs at Columbia. I was most attracted to Dr. Salzman's lab, after reading about his work on positive and negative emotional valence processing in the amygdala. In my coursework in medical school, I learned about the amygdala and its complex role in integrating memory and sensory information, and its connections to cognitive, motor, and homeostatic areas. The Salzman lab seemed to me the perfect place to tackle important issues in neuroscience, with direct clinical applications to psychiatry and the mind-body-world relationship. I set up a meeting with Dr. Salzman, and we quickly developed a good rapport. He was incredibly supportive and encouraging of my desire to pursue doctoral level training, no doubt in part because my story shared considerable overlaps with his own. Emboldened by his encouragement and driven by the exciting work in his lab, I committed to pursuing doctoral training with Dr. Salzman and have been happy with my decision ever since. In many ways Dr. Salzman is an ideal mentor and role model for of the kind of scientist I want to become. In his career, he leveraged his training in the laboratory of Dr. Bill Newsome at Stanford to make major contributions to the areas of visual information processing, decision-making, and emotional learning. Dr. Salzman's work, while not explicitly translational, has provided much-needed groundwork toward understanding the normal physiological functions of the emotional and cognitive systems. Without such grounding, we have little hope of developing satisfying models and treatments for neuropsychiatric pathology. Dr. Salzman has successfully trained eight PhD students and eight postdoctoral candidates, several of whom now work as independent investigators. For these reasons, I have every confidence in my choice of mentor.

**Dr. Stefano Fusi:** Doing meaningful research in systems neuroscience today requires collaborations between theory and experimental work. For this reason, I feel extremely fortunate to be working in the Salzman lab, where my project and many others have originated out of our close working relationship with my co-sponsor, Dr. Fusi, and his group. Dr. Fusi has made numerous contributions toward connectionist models of memory, decision-making, and problem-solving, and in doing so, has inspired much experimental work in our lab and in several major labs around the world.

**Columbia University:** I will echo mathematician Richard Hamming's belief that success comes from apprenticing success. To this end, being a graduate student in Columbia's neuroscience department gives me an ideal training environment, with opportunities to work alongside world-leaders in every sub-field of neuroscience. Of particular significance to me is the strong presence of primate electrophysiologists, prominent leaders in human fMRI studies of cognition, and the singular influence of the Center for Theoretical Neuroscience. I am inspired and humbled to work down the hall from Dr. Mickey Goldberg, who with Dr. Bob Wurtz at the NIH, developed the awake, behaving monkey preparation, and Dr. Larry Abbott, who literally wrote the book on Theoretical Neuroscience. Nobel laureates Drs. Eric Kandel and Richard Axel are regular features at departmental talks, and I have benefited greatly from hearing their questions and observing their though-process. I know that through the guidance of Drs. Salzman and Fusi, amidst the intellectual community of Columbia's neuroscience department, I will be training in an ideal environment to achieve my scientific and career goals.

**RESPONSIBLE CONDUCT OF RESEARCH**

The Columbia Neurobiology & Behavior Program (NBB) offers a course in the Responsible Conduct of Research (RCR), led by Co-Director Dr. Kenneth Miller, that all graduate students take in the 2nd semester of their first year. In addition, all students in their 5th year take a "refresher" discussion based RCR course.

**Format:** The RCR course for 1st-years consists of 14, 1- to 1.5-hour sessions held weekly. NBB Co-Director Kenneth Miller attends all sessions and facilitates discussion. The first 13 sessions are led by an additional faculty member or, for one session, members of Columbia's technology development office. For the last session, the students prepare 1-2 page "op-eds" on a subject of their choice, all students read all of the op-eds before the session, and then in the session (which often runs 2 hours) each student briefly presents their op-ed followed by a general discussion.

I completed the full RCR training course for 1st-year graduate students in the Spring of 2019.

The fifth-year refresher class is led by a professor within the Neurobiology and Behavior Program and is organized as a journal club with student and professors providing and discussing articles of interest around chosen topics.

**I plan to complete the fifth year RCR requirement in the Spring of 2024**.

**Fifth Year Responsible Conduct of Research course description:**

Format and Subject Matter:
This course, which is currently **led by Dr. Michael Goldberg**, features rotating discussion topics including publication ethics, plagiarism, scientific misconduct, limitations of animal experimentation, peer review, and conflicts of interest. In each of four course sessions, the class summarizes and discusses the topics at hand, then applies these concepts to case study examples and holds discussions about them.

The topics of the four 2-hour discussion sessions were, in of Spring, 2020:

1. The ethics of animal research.
2. Scientific conduct within the lab.
3. Communicating science to the world.
4. The ethics of human research.

Duration of Instruction:
The course consists of **four** sessions lasting **2 hours each** for a total of **8 hours of instruction** with Dr. Goldberg.

Frequency of Instruction:
All students take the 1st-year class in their 1st year and the refresher class in their 5th year. I completed the full RCR training course for 1st year graduate students in the Spring of 2019. **I plan to complete the fifth year RCR requirement in the Spring of 2024**.

In addition, I will receive informal instruction on responsible conduct of research from Dr. Daniel Salzman through our private weekly and group lab meetings.

## SPONSOR AND CO-SPONSOR STATEMENT

### 1. Research Support Available

**Sponsor: Daniel Salzman**

| Source | Grant # | Title | PI | Dates | Direct (annual) |
|---|---|---|---|---|---|
| NIH/NIMH | R01 MH082017 | Neurophysiology underlying neural representations of value | Salzman | 4/10/2008 - 10/31/2022 | $397,813 |
| NIH/NIHM | R21 MH116348 | The interactive roles of the amygdala and orbitofrontal cortex during reversal learning | Salzman | 12/24/2018 - 11/30/2021 | $112,500 |
| NIH/NIMH | T32 MH015144 | Research Training in Mood and Anxiety Disorders: From Animal Models to Patients | Roose, Salzman | 7/1/1978 - 6/30/2024 | $371,424 |
| NIH/NIDA | R21 DA045989 | Neurophysiological mechanisms underlying rTMS treatment of addiction | Salzman | 9/30/2018 - 8/31/2021 | $125,000 |
| Simons Foundation | Collaboration on the Global Brain | Neural mechanisms of context dependent cognitive behavior | Fusi, Salzman | 7/1/2017- 6/30/2023 | $243,120 |
| Boehringer Ingelheim | BOERNG 486489 | Columbia Boehringer-Ingelheim Collaboration | Leibel | 11/1/2020 - 10/31/2022 | $399,152 |

These funding sources, combined with my generous Retention Package which is available to support primate research, will ensure that Max's project is fully covered for the rest of his training.

### 2. Previous Trainees

**Sponsor: Daniel Salzman**

Total trainees previously sponsored: 8 predoctoral, 8 postdoctoral. Representative examples:

| Dates | Trainee | Role | Current Position |
|---|---|---|---|
| 2007-2012 | Brian Lau, Ph.D. | Postdoctoral Researcher | Group Leader, Institut du Cerveau et de la Moelle épinière, Paris |
| 2014-2018 | Silvia Bernardi, M.D. | Postdoctoral Researcher | Assistant Professor, Dept. of Psychiatry, Columbia University |
| 2015-2019 | Lee Lovejoy, M.D., Ph.D. | Postdoctoral Researcher | Assistant Professor, Dept. of Psychiatry, Columbia University |
| 2002-2008 | Joe Paton, Ph.D. | PhD Student | Principal Investigator (Faculty), Champalimaud Neuroscience Programme, Lisbon |
| 2004-2010 | Sara Morrison, Ph.D. | PhD Student | Research Assistant Professor, University of Pittsburgh |
| 2011-2015 | Felicity Gore, Ph.D. | PhD Student | Postdoctoral Researcher, Deisseroth and Malenka Labs, Stanford University |

**Co-Sponsor: Stefano Fusi**

Total trainees previously sponsored: 6 predoctoral, 6 postdoctoral. Representative examples:

| Dates | Trainee | Role | Current Position |
|---|---|---|---|
| 2009-2010 | Omri Barak, Ph.D. | Postdoctoral Researcher | Principal Investigator (Faculty), Technion Institute of Technology, Israel |
| 2009-2012 | Srdjan Ostojic, Ph.D. | Postdoctoral Researcher | Principal Investigator (Facutly), École Normale Supérieure, Paris |
| 2005-2010 | Mattia Rigotti, Ph.D. | PhD student | Research Staff Member, IBM T.J. Watson Research Center, Yorktown Heights, NY |
| 2009-2011 | Lorenzo Fontolan, Ph.D. | PhD student | Postdoctoral Researcher, Romani Lab, HHMI Janelia Research Campus |

## 3. Training Plan, Environment, Research Facilities

**Introduction from Dr. Salzman:**
Max's training plan is designed to prepare him for a productive career as a physician-scientist at a major research university. His intellectual and clinical interests in learning, memory, cognition, and the emotions attracted him to my lab where he has become a positive presence and a valuable member of our team. He joined my lab in August 2018 because he knew it would be an ideal environment for his professional development. My lab currently employs three physician-scientists, each of whom splits their time between conducting basic neuroscience research and practicing clinical psychiatry. These lab members have already served as valuable role-models for Max, providing him with both technical and professional advice. As his primary sponsor and mentor, I formally meet with Max one-on-one at least once every week to discuss any results, hurdles or questions regarding his project, and I advise him on topics relating to his broader training goals. I also meet Max at our weekly lab meeting where we conduct a journal club and review any issues in the lab that are affecting productivity or the training environment. Finally, we have numerous informal interactions both inside and outside of the work setting.

**Introduction from Dr. Fusi:**
I have been involved in Max's project since its inception, and I am very happy to be able to serve as his co-sponsor. I have met with Max individually several times to discuss his project, and I will meet with him formally on a monthly basis once he has started data analysis in earnest. In addition, Max often consults with members of my lab on a regular basis for clarification of theoretical principles in machine learning and development of specific computational techniques. It is natural for me to serve as Max's co-sponsor, as his work evolved directly from prior collaborations between myself and Dr. Salzman. Max is talented and very personable, and I am confident he will make tremendous progress in his project and with his research career.

**Training Plan:**
Max is in his third year of the graduate program and has completed all official milestones leading up to this point, which has consisted of academic coursework and successful completion of his oral and written qualifying examination in June of 2020. He is currently in the process of assembling his thesis committee, which will meet every 6-9 months to discuss his progress as well as possible future experiments and analyses. Over the course of Max's remaining time in the doctoral program he will spend the majority of his time on research and drafting manuscripts, with additional time preparing for scientific meetings and teaching. He is working now to draft a review article with a physician-scientist in my lab, Dr. Silvia Bernardi, which we plan to submit for publication by summer 2021. Max and I have discussed publication goals, and we hope to publish behavioral data by 2023. Dr. Fusi and I have worked closely with Max to develop a training plan that will enhance his independence, train him in new technical skills, and refine his scientific thinking and communication skills.

**Skills to Develop:**
Max has mastered many skills that are essential to systems and circuits neuroscience and he will continue to hone them for the rest of his PhD. Specific proficiencies include primate training, and the programming and engineering skills required to design and implement new behavioral paradigms. Max has learned, through discussions with senior lab members and animal support staff, how to successfully train monkeys to perform complex behavioral tasks. In addition, he has gained valuable experience in training naïve monkeys on basic lab activities like pole and collar training as well as simple fixation and saccade tasks. All behavioral tasks developed in the lab are coded using NIH's MonkeyLogic platform, which integrates with MATLAB. Using these tools, Max has learned how to code interactive tasks, which integrate with eye trackers, liquid reward delivery systems, and data acquisition devices. In addition, he has assembled a complete experimental rig in our new lab space, with guidance from Dr. Roberto Gulli, a postdoctoral researcher in our lab, and members of the ZMBBI Advanced Instrumentation Core. Additionally, Max worked with Dr. Lee Lovejoy, another postdoctoral researcher in our lab, to assemble several custom-made optical lick detectors, using an integrated circuit that Dr. Lovejoy designed. As preliminary data has been collected from his project, Max has been developing his quantitative and statistical skills for summarizing and analyzing the data, and he will continue to develop these skills as his project progresses, relating neural activity to behavior. This is an area that both sponsors have considerable expertise in, as do all more senior members of the Salzman and Fusi labs. Thus, Max receives considerable mentoring in this area from both faculty and postdoctoral fellows, in addition to other members of

the Columbia neuroscience community. We are committed to providing Max with training and guidance in the following areas:

**1. Experimental design:**
Being able to design rigorous and creative experiments is an essential skill for any researcher, and graduate training provides the perfect environment to develop these skills. Max has already shown tremendous promise in this area, and he will continue to gain experience and intuition by workshopping his ideas regularly with me and other members of the lab. Furthermore, as Max gains familiarity with non-human primates, so too will his sense of what kinds of tasks are appropriate and feasible for these animals.

**2. Primate electrophysiology and animal training:**
Since joining the lab, Max has had the opportunity to assist postdoctoral researcher Dr. David Barack in his electrophysiological recordings from the monkey orbitofrontal cortex, and will have the opportunity to gain more experience with our collaborator Dr. Silvia Bernardi, as she begins her own recordings in the coming weeks. My laboratory currently uses 32-channel linear V-probes for recording neural activity, often using multiple probes for simultaneous recording from different brain areas. During the course of his experiments, Max will gain further experience with these tools, and will likely have the opportunity to gain proficiency in simultaneous stimulation/ recording experiments through collaborations on other projects in the lab.

**3. Theoretical neuroscience:**
Max recognizes that gaining expertise in computational and theoretical neuroscience is an essential element of his training program. He already has substantial computational skills, which were bolstered through his Introduction to Theoretical Neuroscience coursework in the spring of 2020. In addition, he is a regular participant in weekly seminars in Columbia's Center for Theoretical Neuroscience Theory Center, and has many opportunities to collaborate on his project and others with members of the Fusi lab. He is in an excellent setting to learn from leading researchers in the field, as well as through interactions with the many computationally-minded experimentalists in neighboring labs such as the Churchland and Shadlen labs.

**4. Data analysis:**
Data analysis is central to the scientific enterprise, providing a language with which to quantify the certainty of specific findings. Systems neuroscience in particular is dependent on increasingly sophisticated analytical methods, and Max's project is no different. Max has already mastered core knowledge relating to data analysis through his coursework in Dr. Randy Bruno's Analysis for Neuroscience class, and will continue to practice and extend this knowledge through his doctoral project.

**5. Oral and written scientific communication:**
Science is meant to be communicated, and Max will have ample opportunities to develop his written and oral communication skills through informal presentations in lab meetings, formal talks at scientific conferences, and written communications as part of the grant-writing and publication process. During our lab meetings each week, one person is responsible for presenting a recent or classic scientific paper and leading a discussion. We also have periodic data meetings, where lab members present a progress report of their ongoing work. Max has presented both articles and progress reports, and has always exhibited a masterful and articulate understanding of the concepts and technical details at play. Max will continue to practice and improve his presentation skills in these lab meetings and in thesis committee meetings. He will also present at scientific conferences at least twice per year to gain practice and provide exposure to his work. Prior graduate students in my lab have presented at the national Society for Neuroscience conference, as well as at smaller Gordon Research Seminars, and Max will be expected to do the same in the coming years. In addition, he is required to give a Research in Progress talk for his fellow trainees at the ZMBBI, which he will complete in the 2021-22 academic year. This part of his training plan will culminate when he writes scientific articles for publication in high-impact, peer-reviewed journals. As described above, Max and I share publication goals for review articles in 2021, and new findings by 2023.

**6. Surgical training:**
Max's medical training puts him in a unique position to gain non-trivial expertise in routine surgeries necessary in primate electrophysiology. He will work closely with surgical experts in my lab, including Drs. Lee Lovejoy and Roberto Gulli, to plan, prepare, and conduct implant and craniotomy surgeries for our animals. Gaining these skills will make Max even more competitive for postdoctoral fellowships in the future.

**7. Career planning and guidance:**
Preparing for a successful career that spans both clinical and basic science fields is no straightforward or trivial matter. Max is in an excellent position to gain from the experience of four physician-scientists in the lab (including Dr. Salzman), whom he can regularly consult about issues related to clinical and research training, balancing professional responsibilities, and how best to motivate research questions using clinical insight. Max is not shy, and he has already benefitted from conversations of this sort with these senior investigators.

**Training Environment:**
Max benefits from the rich and collaborative atmosphere at the Zuckerman Mind Brain Behavior Institute located at the new Jerome L. Greene Science Center, which is designed to maximize creative collaboration. Lab members from both of our labs interact regularly with other labs in the building including the labs of Drs. Richard Axel, Mike Shadlen, Mikey Goldberg, and Mark Churchland. In particular, Max has learned a great deal about issues and techniques of computational and theoretical neuroscience by working alongside senior members of the Fusi lab. Our labs also have active collaborations with Dr. Daphna Shohamy in the Dept. of Psychology, who conducts related research into value-based decision-making using fMRI with human subjects.

**Seminars:**
There are multiple seminar series of relevance to Max's training such as the weekly Neuroscience seminar, weekly Theoretical Neuroscience seminar, monthly Zuckerman Institute postdoc seminar, and monthly NeuroLunch seminar. Additionally, a number of distinguished lecture series occur each year, including the Alden Spencer Award Lecture in Neurobiology, and the Louisa Gross Horwitz Award Lecture in Biomedical Sciences, which we encourage all my trainees to attend. In future years, Max will have the opportunity to invite and host researchers from outside Columbia to present at the Neuroscience seminar in their field of interest. He has also benefitted from attending regular seminars hosted by Dr. Salzman and psychiatrist Dr. Steven Roose where guest scientists and clinicians present ongoing research on the biological underpinnings of psychotherapy.

**Research Facilities:**
As a member of the Salzman and Fusi labs, Max has access to the best possible equipment in a newly built laboratory in a new building dedicated to collaborative neuroscience. He has everything he needs to pursue his research, and to do so efficiently and rapidly. Should additional needs arise, we will have no difficulty securing the necessary equipment and resources.

**Salzman Laboratory:**
At the start of 2018, the Salzman lab moved to the newly inaugurated Jerome L. Green Science Center. Space for primate research consists of five experimental booths, located directly adjacent to animal housing for 24 non-human primates and a shared wet-lab space. Additionally, all lab members have a desk in an open-office area which facilitates interactions between the rodent and primate researchers. Max has access to all the necessary equipment and resources he needs to perform the experiments in his research proposal. His experimental rig is newly built and has the best equipment currently available. The recording room itself is a state-of-the-art sound-insulated and RF-insulated recording booth with fiberoptic lighting, creating an ideal low-noise environment. Pertinent equipment available to Max includes: digital recording system with 64-channel amplifier (Plexon), digital neuronavigation system (Brainsight), electrode driver (NAN), and eye-tracking system (EyeLink).

**Surgical Suite:**
Max will perform surgeries in a dedicated primate surgical suite (one of two onsite). This suite is newly built and state-of-the-art. Surgical equipment and instruments were recently purchased and are of the highest quality. Thus, Max has everything he needs to ensure successful surgeries and to gain experience with new techniques and instruments.

**Core Facilities:**
The Zuckerman Mind Brain and Behavior institute hosts multiple excellent core facilities. Our lab regularly makes use of the Advanced Instrumentation Core and MRI facilities (with two 3T Siemens Prisma scanners). Additionally, we use the Virus and Cellular Imaging Cores in our rodent work.

**Animal Care:** Monkeys are housed in a dedicated animal holding room on the same floor as the laboratory, immediately adjacent to the animal prep space and rigs. The animal rooms support paired, group and single housing, with regular access to large play cages. There is additional animal housing elsewhere in the building, devoted to quarantine, overflow, dedicated surgical facilities, and postoperative monitoring. Columbia's Institute of Comparative Medicine (ICM) is fully equipped to acquire, house and care for nonhuman primates. Clinical veterinarian, Dr. Christina Winnicker, directs the Zuckerman Institute Animal Care Facility and a team of veterinarians, veterinary technicians, and animal care personnel are employed in the routine and emergency care of our primates. In addition, the Salzman lab employs a full-time animal technician, Eileen Staufenberg, who has many years of experience in animal care, training and surgical preparation.

**Computing and Software:** The lab has full access to Columbia University's resources including Ethernet and WiFi internet connections, library and reference searches (Medline), and database systems. The Salzman lab has multiple computers for running experiments and data analysis. These computers include licenses for MATLAB, which Max uses for all data analysis, and Adobe Illustrator, which he uses for creating high-quality figures. Additionally, analysis can be accelerated by accessing the Habanero shared high-performance computing cluster managed by the Columbia University Information Technology department. The lab maintains a server and a shared code repository that is version-tracked with Git and it is available to everyone in the lab to use or modify.

## 4. Number of Fellows/Trainees to be Supervised During the Fellowship

**Sponsor: Daniel Salzman**

Pre-doctoral: 3 (including the applicant)
Post-doctoral: 6

The primate research arm of my lab consists of three postdoctoral fellows, two graduate students, and one technician. The mouse research arm of the lab includes three postdoctoral fellows, one graduate student, and two technicians. Together, these members form a talented group with a range of technical and analytical skills. One senior level lab manager oversees the technicians on the mouse and monkey sides of the Salzman lab. In addition, Dr. Salzman co-mentors a postdoctoral fellow jointly with Dr. Daphna Shohamy.

**Co-Sponsor: Stefano Fusi**

Pre-doctoral: 2
Post-doctoral: 11

## 5. Applicant's Qualifications and Potential for a Research Career

Max is an outstanding applicant for the F31 award, and seems destined to make significant contributions to neuroscience and psychiatry as an independent physician-scientist. His determination to advance our scientific understanding of the brain is apparent in every interaction, and is evidenced by the substantial sacrifices he has made to pursue medical and scientific training several years after graduating from college. In personal interactions, Max is always an insightful and critical commentator on the literature, and his questions are never superficial. Our assessment of his potential for a high-quality research career is informed by years of experience mentoring both graduate students and postdocs including postdoctoral fellows outside our labs. Furthermore, we both have extensive experience in the hiring process for neuroscience faculty, which positions us to provide substantial guidance on Max's long-term goal to become an independent investigator in the field. Dr. Salzman has chaired the Neuroscience Department's search committee for new faculty hires every year since 2011, and he has served on the same committee for even longer. He has extensive experience on other search committees as well.

By the time Max joined the lab, he already possessed excellent qualifications. In the two years of his postbac premedical training, he gained substantial experience in a variety of techniques and sub-disciplines in neuroscience, in addition to gaining admission to Columbia's highly selective Vagelos College of Physicians & Surgeons. Although he did not have a traditional background in scientific training, Max nonetheless gained exposure to in vitro electrophysiology, molecular and cellular biology techniques, computational approaches, and clinical research, all of his own initiative. Due to his relatively short time in science, Max has not been able to develop his skills in grantsmanship to a significant degree, but with more experience under our guidance, we

are confident that Max will soon gain mastery over the grant and fellowship writing process. Max's proven record of industriousness is a strong predictor for his future success as a graduate student and eventually as the head of his own laboratory.

Max's growing confidence and skill set have become apparent through his development of this project. Both of us have given Max suggestions for experiments or analyses that he has then refined or changed in order to achieve the best results. Moreover, Max developed the research strategy for this application with minimal guidance. The core ideas are his own and we have merely helped to increase the rigor and depth of his proposal. He will certainly benefit from this quality of independence at every subsequent stage of his career.

In addition to the qualifications and accomplishments outlined above, Max brings a positive energy to the lab and contributes to the liveliness of the department. His personality is warm and expansive, and he has been a major force for social cohesion in the lab, both through his efforts as lab social chair and through his demeanor in daily interactions. He is a team-player who has repeatedly volunteered to help new and junior members of the lab with technical and scientific advice, and he regularly helps with administrative and logistical tasks that other graduate students and postdocs usually fail to notice, let alone attend to. He has also brought this care and energy to the department at large by serving as co-director on the student committee that organizes the open house weekends for prospective PhD recruits, and as a co-director on the student committee that organizes "Boot Camp," a week-long series of lectures, lab tours, and social events to acculturate first-year students to the life in the institution.

In addition, Max has shown a consistent commitment to teaching and mentoring. Graduate students in the program do not have a formal teaching requirement. Nonetheless Max has gone out of his way to serve as a Teaching Assistant for the undergraduate introductory course in cellular and molecular neurobiology, and established a special journal club over the summer of 2019 to introduce rotating undergraduates to the major papers from our lab. From conversations and direct observation, Max clearly takes great pride in his teaching. He feels it is a professional responsibility to help guide and encourage young scientists – a sentiment he no doubt brings to his clinical training environment as well. Max has explained that teaching also helps him to deepen his conceptual understanding of difficult topics, in part by formulating explanations in a variety of ways for different students. Max's career goal to become a principal investigator will no doubt be facilitated by his enthusiastic engagement with teaching and mentoring, in addition to his strong research activities.

The training plan we have outlined for Max is consistent with his goals and our views concerning the essential skills needed to be become a high-quality, independent researcher. His impressive technical, analytical, and professional skills make him an extremely competitive applicant for the F31 award. We look forward to observing the ways in which his doctoral research will advance our understanding of the relationship between neural representations and complex, flexible behavior.

## DESCRIPTION OF INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING

***Structure of the Program***

The Neurobiology and Behavior Graduate Program provides broadly-based training across neuroscience from molecular biology to theory. Students enter their Ph.D. studies in our program from a wide variety of backgrounds. The aim of the doctoral curriculum is to equip all students with the knowledge base and professional skills required for completion of Ph.D. research and in preparation for diverse career trajectories after the doctoral degree is awarded.

The program currently has 129 faculty mentors, over 100 graduate students, and spans three research campuses (Arts and Sciences, Columbia University Medical Center, and Manhattanville). The program is co-directed by Darcy Kelley, Ken Miller, Wes Grueber and Ai Yamamoto. Program funding is provided by the Department of Neuroscience, NIH training grants, and institutional support. In addition, students apply for, and are awarded, individual fellowships including NSFs, DODs and NRSAs. Program policies are reviewed by two independent committees. One consists of an external advisory committee of three senior faculty members from peer institutions with extensive Ph.D. training experience, who meet every 5 years. The second committee is an internal advisory committee that meets twice a year, and is made up of both Columbia faculty members with mentoring experience in the program and advanced graduate students. A student advisory committee seeks input on the program from students and provides input to the internal advisory committee and Co-Directors.

A number of committees are involved in planning and carrying out program activities. Most of these committees include both faculty and student representatives. These include an Executive Committee that appoints the Co-Directors, a Student Progress Committee (faculty mentors), an Admissions Committee, an Open House Committee (Graduate Recruiting), a Curriculum Committee, a Seminars Committee, and a Retreat and Poster Show Committee. Participation is broadly distributed among faculty members and students.

An internal program website (wiki) is maintained with comprehensive program information (including forms, student and faculty profiles, course lists and syllabi, funding, outreach, and career resources, etc.). The program's dedicated administrative support consists of Alla Kerzhner, Program Coordinator, and Rozanna Yakub, Administrative Coordinator. Additional support is provided by the central administration of the Department of Neuroscience along with other centralized administrative and student services offices.

***Milestones***

***Year 1 milestones:*** Courses: All entering students attend the Neuroscience Boot Camp in August and take Analysis for Neuroscientists (statistics and MATLAB instruction; Fall) and Responsible Conduct of Research (Spring). Students also give talks on rotations at the beginning and end of the Spring semester. All students take Survey of Neuroscience (Fall) and Developmental and Systems Neuroscience (Spring). Each entering student consults with a Program Co-Director before classes begin to plan an academic schedule. Co-Directors teach or co-teach first year courses each semester and thus interact with students extensively upon entry into the program. In year 1 students applying for Soros, DOD and Hertz fellowships receive one-on-one tutorials.

Research rotations: In year 1, students engage in a series of laboratory rotations, typically three, in order to choose a thesis research lab and to gain experience in techniques or computational approaches. Students are strongly encouraged to choose their research mentor by the end of June of year 1, though students occasionally do a fourth rotation before choosing a mentor.

***Year 2 milestones:*** In Year Two, students complete two additional advanced courses and prepare and defend a qualifying examination by the end of May. Some students take additional courses and many choose to gain teaching experience (not required) in the cellular or systems neuroscience undergraduate courses or the theoretical neuroscience graduate courses. Prior to the qualifying exam, students prepare a specific aims page, and provide this to the Co-Directors along with a list of possible qualifying exam committee members. Upon approval, the students assemble the committee, set a date for the exam (with administrative assistance), and write and distribute a 6-page NIH-style research proposal. This proposal forms the basis for discussion during their qualifying exam. At the exam, the committee assesses the student's grasp of the literature both in their field and more generally in neuroscience. The committee may recommend additional preparation as the result of this examination, but failure is exceedingly rare (1 student in 20 years).

Upon completion of the qualifying exam, the students assemble a thesis committee consisting of four faculty members including their thesis advisor. Thesis committee meetings are held yearly. Starting in year five, meetings are held every six months, and three months before the defense the committee meets for a final time to re-review the thesis and approve the defense.

***Years 3 - 6 milestones:*** Students defend a doctoral thesis based on their original research. The average time to degree in the program over the past 10 years is 5 years, 7 months.

***Additional milestones***: In Years 3-6 students take Professional Skills for Neuroscientists, which is taught by program faculty, professionals in science allied fields, and program alumni. Course topics include: (1) Navigating graduate training (choosing rotations, choosing a thesis lab, preparing a qualifying examination and NRSA application -writing workshop, when to defend, choosing faculty committees), (2) Communicating science (giving a research talk, writing a Ph.D. thesis, grant writing, communicating to a lay audience, preparing a scientific paper, reviewing scientific papers, grant and other proposals), (3) Beyond the Ph.D. (post-doctoral training for a research career, securing a faculty position, scientific publishing, science policy, biotech/pharma, NIH and NSF scientific review, intellectual property/tech transfer, work/life issues), (4) Responsible conduct for advanced graduate students (4 biweekly sessions specifically required for advanced students). Beginning with the first thesis committee meeting, each student creates an individual development plan, which then serves as a template for discussion with the thesis mentor and the thesis committee. The program has no formal teaching requirement, but opportunities are available for teaching or TAing undergraduate and graduate level courses.

Neurobiology and Behavior graduate students present their research in a Research in Progress seminar series, which is attended by other students, postdocs, and program faculty. Typically, this presentation occurs in year 3 or 4. Two students present each month and talks are 20 minutes each, with discussion and feedback during and after the presentation. In preparation for their talks, students participate in a nanocourse, Presentation Skills for Neuroscientists, led by program faculty.

***Monitoring, evaluation:*** Progress toward the PhD degree is monitored throughout the graduate years via several mechanisms. Overall **progress in the program** is assessed and conveyed to early stage students during a yearly meeting with a program Co-Director together with the current rotation advisor. Since Co-Directors are very familiar with both courses and rotations, the feedback at this time is personalized and focused on identifying a suitable lab for thesis research, and possible deficiencies in coursework. During selection of rotation labs and proposal preparation students meet more frequently with a Co-Director. First-year students are in weekly contact with Co-Directors via required courses. **Progress through rotations** is monitored by Co-Directors during the three rotation talks, and by rotation mentors. Students present their rotation work and rotation experiences to Co- Directors and other first-year students.

**Progress in the ability to develop and defend a research project** and general scientific proficiency are monitored during the 2nd year by the research mentor, the qualifying exam committee and the student progress committee. **Progress thereafter** is monitored by research mentors, the thesis committee, and Co-Directors. Students complete and submit a yearly progress report to the program Co-Directors, which includes courses taken, thesis committee meetings, meetings attended, outreach and teaching activities, and program feedback. The frequency of thesis committee meetings and the committee's recommendations are shared between Co-Directors. If there are impediments to progress, such as delays in the qualifying exam, gaps in thesis committee meetings, or changes in thesis research, these are identified and addressed by the thesis committee, in consultation with the Co-Directors. The mentor may also report problems at any time to program Co-Directors.

***Applicant's Progress:*** Max Pensack is in excellent standing in the Neurobiology and Behavior graduate program. He has completed all required coursework appropriate for his standing in the program, and successfully sat his qualifying exam with Drs. Stefano Fusi, Elias Issa, and Michael Goldberg in June 2020. In 2018, Max attended the biennial Neurobiology and Behavior Program retreat and in 2019, he attended the annual Cognitive Computational Neuroscience conference in Berlin. He will present his work to graduate students and faculty in our Graduate Student Research in Progress meeting in the coming years. He is active in scientific outreach and education as a classroom volunteer in the Columbia University Neuroscience Outreach (CUNO) program and as an elective teaching assistant in the fall 2019 undergraduate Cellular and Molecular Neurobiology course. Additionally, he organized an undergraduate journal-club within the Salzman lab for rotating students in summer 2019. Within the program, he has served on the doctoral program's new student recruitment committee, has been heavily involved in new-student orientation, and has been a vocal participant at Internal Advisory Committee meetings. The above information was provided by Wes Grueber, Darcy Kelley, Ken Miller, and Ai Yamamoto (Graduate program Co-Directors).

# PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

**Use of Human Specimens and/or Data**

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

## VERTEBRATE ANIMALS

**1. Description of Procedures:** All of the experiments outlined in this proposal will be performed using adult rhesus monkeys (*Macaca mulatta*), male, weighing between 5 and 15 kg. The monkeys will be Herpes B-, and as many as possible will be specific pathogen free. At least 2 monkeys will be used for electrophysiological recordings in this grant, as is typical in electrophysiological studies. The full experimental protocol has been approved by the Columbia University Institutional Animal Care and Use Committee, and that protocol is in compliance with the NIH Guide for the Care and Use of Laboratory Animals. The general procedures used are standard techniques for electrophysiology in awake, behaving monkeys. They have been developed over several decades in multiple laboratories and are described in numerous publications in the scientific literature. These techniques are constantly being refined in order to minimize both the number of animals and any possible causes of discomfort.

The neural populations required for the analysis are large, and it is important to verify our findings in multiple animals. Note that for this type of work, male monkeys are typically employed because they are more readily available for purchase, therefore meaningful comparisons of findings across gender are not possible.

Monkeys will be trained on the serial reversal-learning task (holding or releasing buttons according to visual stimuli displayed on a computer monitor; see: Research Strategy) and are operantly conditioned using water or juice as a reward. When an animal is in experimental use, it works in the context of an experiment or training session five days per week. At the beginning of the day, the monkey is led from its home cage using a pole and collar handling procedure. The monkey is weighed and then seated in a primate chair, and then works on its task for 1-6 hours depending upon the demands of the experiment or training regime. Although the monkey is restrained during this time, the primate chair is adjustable along several dimensions so that the animal can remain comfortably seated. At the end of the experiment, the monkey is returned to its home cage. After several days of training, these procedures become routine for the animals and for the investigator. Gentle handling and common sense go a long way toward establishing a good working relationship with each animal. It is in the best interest of the animal *and* of the investigator to create a calm, nonthreatening environment for the animals.

Training is accomplished by conditioning techniques using positive rewards for desired behavior. Our animals work for liquid reward (water or juice), and we therefore control water intake carefully. A minimum daily level of fluid intake for adequate hydration is established individually for each animal (judged during training), and supplemental fluid is given if the animal does not obtain its minimum during the experimental session. Extra fluid is provided on weekends. Hydration levels are monitored daily for each animal by measurement of body weight, assessment of skin turgor, and visual inspection of feces. These indicators are occasionally augmented by measurements of urine specific gravity. Daily records are kept monitoring the monkey's fluid intake and health status. We are careful to maintain our animals in a healthy but motivated state.

Veterinary Care: The monkeys will be housed, and the experiments performed, in the Gerome L. Greene Science Center (JLG). Dr. Christina Winnicker is the veterinarian responsible for the vivarium at JLG, which has received unrestricted AAALAC certification. A clinical veterinarian (Dr. Winnicker or her colleagues) is on call 24 hours a day, and specialists are available for consultation. The veterinarians have been very attentive to the needs of animals in the Salzman lab. Daily husbandry is in the hands of trained employees of the animal care facility but is supervised by Dr. Salzman and other members of the Salzman lab. Dr. Winnicker oversees a colony of over 50 Rhesus monkeys, used by a number of investigators. She has years of experience with monkeys with head implants. The monkeys are observed by a veterinarian every working day. A number of procedures are used to ensure the psychological wellbeing of the monkeys, the most important component of which is pair housing. The monkeys are housed in Primate Products cages that allow pair acclimation, first through Plexiglas dividers, then through grooming bars. If the monkeys are compatible with each other, then the dividers are removed and the monkeys are allowed to be paired in the same space. It has been the PI's experience that pair housing is possible for many monkeys. With appropriate care and attention, implanted monkeys can be paired with un-implanted monkeys, and monkeys on controlled access to fluids can be paired with monkeys who are not restricted, so long as the husbandry staff has been adequately informed about these issues. The pair housing of monkeys is monitored on an ongoing basis, and any sign of difficulty (conflict) can result in our separating a pair. Each pair of cages has access to a double height activity module, and each pair

or each individual (depending upon housing status) are allowed into the activity module for half day sessions several times a week. Other maneuvers to provide psychological wellbeing for monkeys include primate puzzles and toys that are changed frequently.

Surgical Procedures: The experiments involve multiple survival surgeries. After preliminary training, monkeys will be operated on for the implantation of head holding devices. The implantation of a recording cylinder usually comes at a later surgery after extensive training on the emotional learning task. The surgical team will be wearing sterile clothing and using sterile instruments. Anesthesia will first be induced with ketamine and diazepam, after which animals will be intubated and then maintained with isoflurane. Animals will have respiratory, blood pressure and EKG monitors, and be placed on a heating pad throughout the period of anesthesia. The surgical site will be prepared by removing any hair from the area with clippers followed by a depilatory (Nair, CaOH) if necessary. The area will then be scrubbed with Betadine a minimum of three times, washed with 70% EtOH and then painted with Betadine solution. The animal is then placed in a stereotaxic device to ensure the correct orientation of the head and to facilitate the correct placement of implants: the head holder and recording cylinders, which are made of high-grade stainless steel, titanium or a copolymer resin (20% glass-filled Delrin 570). In practice, the recording cylinders are usually placed during a later surgery, using the same procedures described here. A midline incision will be made through the skin over the cranium extending from the region of the external occipital protuberance to within a centimeter or two of the brow ridges. If necessary, the temporalis muscle will be reflected or partially removed to gain access to the underlying periosteum. The periosteum will then be scraped away and the bone cleaned and dried. When placing cylinders, a trephine will be used to remove a circular disc of bone from the cranium, taking care to ensure that the underlying dura is left intact. Cranial implants will be held in place with one of two methods: 1) a titanium headpost with 2-4 adjustable strips will be positioned stereotaxically, and the strips screwed to the skull; or 2) a dental acrylic (methyl methacrylate) cap will be secured to the skull with numerous self-tapping titanium or ceramic cortical bone screws. After a final cleaning with saline and/or hydrogen peroxide, the periosteum will be dried and may be painted with cyanoacrylate (Vetbond). A stainless steel or titanium plug (used to secure the head to the primate chair during experiments) will be positioned stereotaxically and then secured to the skull with dental acrylic, which bonds together all of the screws/bolts/strips to form a single robust implant. Recording cylinders will be positioned over the trephine hole stereotaxically and secured to the rest of the implant with dental acrylic, or, in the case of the titanium headpost, four nylon screws will be implanted in the skull using key slots, and the cylinders bonded to them by acrylic. The wound edge will be cleaned and sutures inserted to keep the skin closely apposed to the implant until healing has occurred. If necessary, modified V-plasty incisions will be used to ensure that the skin is closely aligned with the margin of the implant.

Postoperative care will include daily monitoring and cleaning of the wound using Betadine solution and antibiotic ointment for a period of 10-14 days. Sutures will be removed 7-14 days after the surgery. Buprenorphine (0.03mg/kg BID, typically for 2 days) or other analgesics will be used postoperatively to alleviate pain. In addition, acetaminophen (20-30mg/kg) may be used for minor pain. Cefazolin (25mg/kg) and gentamicin (2-4mg/kg) are given postoperatively to prevent infection.

**2. Justifications:** We use rhesus monkeys for several reasons. First, rhesus monkeys adapt easily to the behavioral training situations employed in the laboratory. They train well, are calm while sitting in a primate chair, and will perform steadily for several hours during daily experimental sessions. Secondly, Dr. Salzman has extensive experience in locating and recording from brain areas in rhesus monkeys. It therefore is prudent to continue with this species since the success of the research depends critically on our ability to place microelectrodes in the appropriate brain areas. Many behavioral experiments have shown that the macaque brain systems mediating higher functions serve as an excellent model for the equivalent human systems. Thus, our findings are likely to be directly applicable to understanding cognitive and emotional processes in humans. Besides, as highlighted in the research component of this grant, anatomical homologies are essential when translating therapeutic interventions and it is well known non-human primates' anatomy is more homologous than rodents to the human brain. In this grant in particular, we focus on hippocampus and prefrontal areas. Monkeys, unique among non-human mammals in possessing a well-developed internal granular layer in prefrontal cortex, provide a key intermediate step for the development and understanding of circuit based therapies in humans.

**3. Minimization of Pain and Distress:** Three situations exist in which the monkey might experience discomfort, distress, and/or pain: a) Survival surgery, b) Restraint for handling or routine testing, and c) Minor procedures and care of implants. Additionally, we outline our procedures concerning d) Euthanasia and humane endpoints.

a) Survival surgery: These are accomplished using standard surgical techniques and are absolutely necessary since none of the proposed experiments can be performed without them. All surgical procedures are performed under surgical anesthesia and aseptic conditions in an animal surgical facility administered by Dr. Christina Winnicker, veterinarian in the JLG animal care facility. Additional analgesia will be provided if warranted and recommended by veterinary staff.

b) Restraint for handling or routine testing: The animals live in squeeze cages that can be used to administer injections. Restraint for certain procedures, such as TB testing, is accomplished with ketamine. Physical restraint of monkeys is essential during the experiments themselves in order to protect delicate equipment and microelectrodes that are affixed on or near the monkey's head, as well as to permit the measurement of eye position via infrared camera. During experiments, therefore, monkeys are seated in a custom-built plexiglass chair that is tailored to meet the size of each animal. The chair allows the monkey to move his body freely during an experiment, while the orientation and stability of his head are maintained via a headpost attached to the chair. Monkeys are never transported to or from the laboratory under head restraint. Overall, the monkeys do not find sitting in the chair aversive. They quickly come to associate the chair with the expectation of positive rewards, and often assist with the daily placement of their headposts. The end of each experimental session is in large part dictated by the monkey: when the animal quits working, the experiment ends for the day and the monkey is returned to its home cage.

c) Minor procedures and care of implants: The wound margin will be cleaned every working day and will be treated with bactericidal agents such as dilute Betadine or chlorhexidine. If excessive scabbing occurs at the wound margin, minor debridement may be necessary. Margin cleaning, rinsing of the recording chamber, and implantation of recording electrodes can be accomplished with little or no discomfort to the animal, as evidenced in the animals' behavior. Although rare, analgesics are administered when necessary under the supervision of the veterinary staff.

d) Euthanasia and humane endpoints: At the end of the current study, our preferred outcome is that monkeys will be employed for use in other studies in the lab. When animals are not suitable for continued experiments, we will prioritize sending them to primate sanctuaries in lieu of euthanasia – a practice we have initiated successfully in recent years. However, occasionally animals must be euthanized in order to conduct histological studies or to reconstruct recording sites. Our method of euthanasia is consistent with the *American Veterinary Medical Association Guidelines for the Euthanasia of Animals* and will always be performed by a licensed veterinarian to keep any pain or distress to the animals to an absolute minimum.

## RESOURCE SHARING PLAN

**Data Sharing Plan:** The data collected through the proposed experiments will be disseminated via publication in peer-reviewed journals or provided to the NIH. Every effort will be made to select open-access journals in order to increase public availability of the findings. All data and analyses will be freely provided to qualified investigators within the scientific community, as replication and accountability are necessary for scientific progress. In addition, any code that is deemed to have broad scientific use will be made available on the laboratory website.

**Sharing Model Organisms:** Every effort will be made to share monkeys across several projects in the lab. Occasionally, this will not be possible, as some projects require histological studies after the completion of behavioral and electrophysiology experiments. Any information regarding specific experimental animals used in this study will be made available to qualified investigators upon request.