OMB Number: 4040-0001
Expiration Date: 10/31/2019

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: 04-23-2019

Animal Welfare Assurance Number A3539-01

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: Eligible to be designated; project will have no impact.

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*	02_Project_Summary1011499127.pdf
8. Project Narrative*	03_Project_Narrative1011499007.pdf
9. Bibliography & References Cited	04_References_cited_11011499128.pdf
10.Facilities & Other Resources	05_FACILITIES_AND_OTHER_RESOURCES1011499009.pdf
11.Equipment	06_EQUIPMENT1011499010.pdf
12. Other Attachments	BRAIN_Relationship1011499011.pdf Diversity_Eligibility_Ltr1011498990.pdf

Project Summary

Social deficits are common in psychiatric disorders and available treatments are limited. Our lack of basic knowledge on how the brain controls social behaviors makes it challenging to develop therapeutics for social deficits. For numerous animal species, social rank dictates many aspects of behavior, such as access to resources and resilience to stress. Individuals with higher social rank typically win more often during social conflicts (e.g. food competition) and show more agonistic behaviors; collectivity referred to as dominance behaviors. Cross-species evidence suggests that the medial prefrontal cortex (mPFC) plays an important role in social dominance. However, exactly how the mPFC encodes social rank and which mPFC inputs and outputs contribute to dominance behaviors is unknown. Multiple studies show that the ventral hippocampus (vHPC) is necessary for social memory, and more recently, a study showed that this role involves vHPC input to the mPFC. Furthermore, preliminary data suggest that the projection from the mPFC to the lateral hypothalamus (LH) modulates social dominance behavior. These findings in combination with the literature suggest a model in which the mPFC receives social memory information from the vHPC and guides social behaviors via modulation of LH GABAergic and glutamatergic subpopulations. Progress in uncovering neural correlates of social behavior has been limited by the tools used to characterize murine social behavior, since existing social assays lack trial-structure needed for statistical power and common measurements of social behavior are simplistic (e.g. sniffing). Overcoming this challenge required developing a trial-based social competition assay in which mice compete against cagemates for a reward signaled by a tone. Due to its trial structure, this assay facilitates the quantification of social behaviors and subsequently the identification of neural correlates for social dominance. In this assay, dominant mice win most of the rewards across trials, occupy the reward port and displace mice from the reward port more often than subordinates. Machine learning approaches will be used to profile the behavioral differences seen across social rank during the competition assay. Utilizing this ethologically relevant social competition assay, circuit manipulations, in vivo neural recording methods and machine learning allows testing the hypothesis that the tripartite vHPC-mPFC-LH circuit encodes social dominance. Altogether, this research will provide a new approach to study social dominance and will further our understanding of how the distributed circuits of the mPFC modulate social behavior. Furthermore, pinpointing the neural circuits underlying social behaviors will facilitate identification of potential therapeutics for social deficits in psychiatric disorders. Finally, completion of this research will provide training opportunities in statistical approaches for behavioral analysis and new circuit dissection tools, which are essential for the candidate to become an expert in social neuroscience and to start a successful independent research program.

Project Narrative

Social behavior deficits are key features of many psychiatric disorders, yet little is known about how the brain controls normal social behaviors. I propose to dissect the role of the medial prefrontal cortex circuits in social dominance by combining wireless electrophysiology, calcium imaging, optogenetics and machine learning. This study will broaden our understanding of how neural circuits control normal social behaviors and will contribute to the identification of novel interventions for social deficits.

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FACILITIES AND OTHER RESOURCES

The Tye lab is located at the Salk Institute for Biological studies, an outstanding environment for the proposed training program. The Salk Institute has state-of-the-art facilities and a thriving research community that includes 65 faculty members with a wide range of expertise including molecular and cell biology, systems neuroscience, and advanced computational analysis of learning, memory and behavior. The highly collaborative environment will provide a wealth of support for Dr. Padilla-Coreano in her research and professional development during the mentored phase of her K99/R00 training. All Salk Institute facilities will be available to provide expert support, assistance and equipment to ensure the success of Dr. Padilla-Coreano's research.

Laboratory and Office Space: The Tye lab occupies 4132 ft^2 of space on two floors at the Salk Institute. The lab is fully equipped for optogenetics, behavioral experiments, *in vivo* electrophysiology, whole-cell patch clamp recordings, surgeries, and all other procedures described in the research proposal (*see also 'Equipment' section*). The lower floor of the lab is located adjacent to the animal housing facility, allowing mice to be easily transported for behavioral experiments with minimal distress. Dedicated space for Dr. Padilla-Coreano includes a personal desk and two-monitor PC for data analysis with GPU. Dr. Padilla-Coreano also assisted in the design of the current Tye lab space; she was involved in planning meetings prior to the lab's relocation to the Salk Institute. As such, all areas are ideally configured for her research, including a dedicated behavioral room designed specifically for *in vivo* electrophysiology in electrically-shielded operant chambers.

Animal Facilities: The *Animal Resources Department* supports all Salk faculty and students in their research with world-class facilities for animal research, including over 45,000 ft^2 of vivarium space. The Tye lab's colonies will be housed in a room on reverse light/dark cycle, with space for over 700 cages of mice. Expert veterinary staff will provide monitoring and care for experimental animals, and be available for consultation on any issues that may arise. All mouse breeding, genotyping and husbandry will be done by facility staff, maximizing Dr. Padilla-Coreano's time available for research. These staff will also provide Dr. Padilla-Coreano with any necessary additional training in surgical or behavioral procedures, and ensure that all experiments are conducted in accordance with appropriate ethical and safety standards. The Salk Institute has been a leader in animal research, and all facilities are accredited by AALAAC and adhere to NIH standards for animal welfare.

Core Facilities: One of the highlights of the Salk Institute's research environment is the wide array of outstanding core facilities located there. These are independently funded and staffed by expert scientists, providing both equipment and expertise to all members of the Salk research community. While the Tye lab is fully equipped for the proposed experiments, several of these core facilities have expertise directly related to the present research project and will be an invaluable resources for any challenges or alternative directions required in Dr. Padilla-Coreano's research. The *Biophotonics Core Facility* is equipped with cutting-edge imaging and microscopy equipment for analysis of biological samples, and will be consulted for assistance with any imaging needs arising in the project. Instruments in the core include a Zeiss LSM710 Laser Scanning Spectral Confocal with inverted microscope equipped with 405, 488, 514, 561, 594 and 633nm lasers, and a Spinning Disk Confocal with 405, 488, 561 and 639nm lasers. This equipment will ensure any imaging needs beyond the Tye lab's existing confocal imaging suite will be easily accommodated. The *Next Generation Sequencing Core Facility* provides cutting-edge genomics technology for rapid sequencing of biological tissue, and will be available for any investigation of neuronal gene expression which arises during the proposed experiments. This facility also works in conjunction with the Salk Institute animal facility to ensure rapid and accurate genotyping of all transgenic mouse lines used in research. The *Crick-Jacobs Center for Theoretical and Computational Biology*, a multidisciplinary bioinformatics core, will be able to assist with any additional computational analysis needed for Dr. Padilla-Coreano's work. Finally, the *Viral Vector Core* is a world leader in the development of viral vectors for use in neuroscience research. They will provide design and production services for any additional viral constructs required for the proposed experiments, as well as training in the safe and effective use of these technologies in research. Dr. Edward Callaway, a member of Dr. Padilla-Coreano's supervisory committee, also works closely with the viral core staff scientists, and is one of the pioneers in developing new viral vector tools for use in neuroscience research.

Computation Resources: The Tye lab has over 40 PCs and laptops for data and behavioral analysis,

including a personal computer solely for Dr. Padilla-Coreano's use. The lab has also recently constructed two high-end analysis computers with specifications designed for computationally-intensive neural network applications, which will be used for behavioral analysis in the present study. Extensive support for her computational goals will also be provided by Dr. Terry Sejnowski and his lab members, a member of Dr. Padilla-Coreano's mentoring committee, who is an expert in machine learning approaches (*see also Dr. Sejnowski's Letter of Collaboration*). Dr. Padilla-Coreano will also have access to further training in computational approaches through courses and workshops provided by the University of California San Diego (UCSD), with which the Tye lab is affiliated, including seminars and symposia organized by the UCSD Institute for Neural Computation (INC).

Environment: The Salk Institute is dedicated to providing postdoctoral research associates with a variety of professional development opportunities outside of the laboratory setting to promote successful transitions into independent research positions. In recognition of this, Salk recently established a dedicated Postdoctoral Office to provide support to the career and professional development of postdoctoral trainees. The Postdoctoral Office provides resources and support in a variety of areas including supporting and enhancing existing career development programs and identifying strategies and tools to enhance the postdoctoral experience. The Postdoctoral Office sponsors events that are tailored to the development of Salk postdoctoral fellows. Departmental websites contain numerous links to external resources concerning grant writing, scientific networking, and career development. They also include information pertaining to Individual Development Plans, which are intended to enumerate both short- and long-term career goals and help monitor progress toward those goals.

Additionally, the Salk Society of Research Fellows (SRF) is a volunteer group of graduate and postdoctoral trainees whose goal is to foster a sense of community among San Diego researchers and to organize career-development activities. Programs coordinated by SRF include:

- The "Salk Featured Fellow" program – a monthly session that allows Institute fellows to present recently published research to the group.
- The "Coffee with a PI" series allows small groups of fellows to spend the morning with a Salk faculty member, thereby fostering collaborations and expanding the mentorship base for participating postdocs.
- Competitive travel award selections for trainees to attend meetings and present their work.
- Leadership opportunities as a rotating Committee Chair.

To complement these efforts, Salk has partnered with neighboring institutes to form the Torrey Pines Training Consortium (TPTC). The TPTC committee contains at least one representative from each of the following: the Academic Services Department at Salk, the Office of Postdoctoral and Visiting Scholar Affairs at the University of California, San Diego, the Career and Postdoctoral Services Office at The Scripps Research Institute, and the Office of Training and Academic Services at the Sanford Burnham Prebys Medical Discovery Institute. The TPTC committee meets on a regular basis to plan upcoming events, to identify training opportunities (both internal and external), and to address gaps in postdoctoral training resources. Events sponsored by the TPTC include, but are not limited to:

- Academic Leadership Symposium – An intensive 2-day course on laboratory management with sessions based on the highly successful BWF-HHMI Course in Scientific Management for the Beginner Academic Investigator. The course is designed to equip attendees with the knowledge and professional competencies to lead innovative and productive research programs. It is typically offered every 12–18 months.
- Funding Fest – A month-long event that consists of multiple workshops and seminars (typically 5–7). Commonly addressed topics include: Basics of Scientific Grant Writing, Pathways to Independence–Winning an NIH K99/R00 Grant, and Identifying Funding Opportunities. Funding Fest is an annual event that usually takes place in the spring.
- Career Building Seminar Series – Seminar speakers are invited throughout the year to give talks on career-related topics. Previous talk titles include: The Academic Interviewing Process, Making the Most out of Your Presentation, and Structuring Your Scientific Paper.

Institutional Support: Since it was founded in the 1960s, the Salk Institute has been one of the world's preeminent research institutions, with an international reputation for leadership in all areas of the life sciences. All core facilities are well-supported by independent funding, and in 2017 financial support from private donors and competitive government grants to Salk Institute scientists totalled over 140 million dollars, providing

enormous support for the cutting-edge research which is the institute's mission. Training the next generation of leaders in scientific research is also a major priority for the institute. Trainees are supported with competitive travel awards to present their research, mentoring programs with senior faculty members, and a Career Building seminar series which provides training in topics such as academic job interviews, presenting scientific research and manuscript preparation, among many others. Over 2500 scientists have been trained at Salk, and past trainees have gone on to become internationally recognized leaders in their respective fields, including 5 Nobel Laureates. Dr. Padilla-Coreano will have the full support of the institute during her proposed research program and as he transitions to an independent research position.

All resources of the Tye lab and the Salk Institute will be fully accessible to Dr. Padilla-Coreano in her research, and provide outstanding support for her training and career development.

EQUIPMENT

All equipment required for the proposed experiments is currently present in the Tye Lab at the Salk Institute for Biological studies, and has been used by Dr. Padilla-Coreano to produce preliminary data.

General: The Tye lab consists of 4,132 square feet of newly-renovated lab space in the Salk Institute for Biological Studies. The lab contains a complete wet lab with all equipment for histology, including a Leica VT1000 microtome, bench-top centrifuges, a cold room, three -20°C tissue freezers, two -80°C freezers for storage of viral vectors and other reagents, a pH meter and a water bath.

Stereotaxic surgeries: The lab has a dedicated surgery area with six digital stereotax apparatuses (dual arm for implanting two optic fibers simultaneously), each with an isoflurane machine, arm-mounted microscope, and motorized UltraMicroPump III microsyringe with Micro 4 Controller for controlled injections of viral vectors.

Behavioral equipment: The lab has 16 operant chambers (Med Associates) which can be run by one of three independent Med-PC control units. Each chamber is enclosed in a sound-attenuating cubicle with conductive carbon fiber matrix to act as a faraday cage for electrically shielding electrophysiology recordings. Operant chambers are equipped with detachable shock floors that have custom-built TTL-controlled relays for reducing electrical noise, reward ports with LED beam detectors, multi-tone capable speakers, white noise generators, and infrared-capable cameras interfaced with a separate computer for high-resolution video recording of behavior. Three dedicated rooms for larger behavioral tasks are equipped with ceiling-mounted cameras, licensed Noldus Ethovision software for real-time mouse tracking and triggering of optogenetic stimulation, and test apparatuses for open field, elevated plus maze and real-time place preference experiments.

Optogenetics: The lab is fully equipped with all necessary equipment for the use of optogenetics including fiber cutters, motorized fiber polishers and light meters for the manufacture of optic fibers. The lab has 16 473-nm lasers and 8 593 nm lasers, Master-8 controllers for regulation of light pulse width and frequency, and 26 commutators with fiber optic rotary joints for light delivery in freely-moving experiments, including 14 dual commutator 'splitters' which allow simultaneous delivery of light to two optic fibers in bilaterally implanted mice.

***In vivo* electrophysiology:** The lab is fully equipped for *in vivo* electrophysiology, with 12 OpenEphys acquisition boards and 22 Intan headstages for recordings. In addition, we are equipped with wireless *in vivo* electrophysiology systems from Spikegadgets LCC. A dedicated area is equipped with all materials required for custom electrode and optrode fabrication including two microscopes (Leica), an impedance meter and current generator for gold-plating electrode tips. There are multiple licensed copies of Plexon Offline Sorter and NeuroExplorer software for data analysis, as well as custom-built MATLAB-based tools for analysis of single-unit neuron activity. The lab also has custom-built Arduino-based controllers which interface with acquisition boards for time-locked laser light delivery in joint optogenetics/electrophysiology experiments ('phototagging').

***Ex vivo* slice electrophysiology:** The lab has two complete whole-cell patch clamp rigs for work with *ex vivo* slices. Each rig is equipped with a Multiclamp 700b microelectrode amplifier and a Digidata 1440a low-noise data acquisition system controlled by a dedicated PC, a faraday cage, manipulator arms, a perfusion chamber, Master-8 controlled LEDs for optogenetic stimulation experiments, and an Olympus BX61 microscope and Scientifica SliceScope Pro 2000 for imaging of both fluorescence and DIC microscopy.

Imaging: The lab has an Olympus FV1000 confocal imaging system equipped with 405nm, 488nm, 543nm, and 633nm lasers, with a motorized stage and software packages for 2D and 3D reconstruction of brain slices. The lab is equipped to process whole brains using the CLARITY method, and has a BZ-X800 Slide Scanning Microscope (Keyence) for verification of viral vector expression and electrolytic lesion targeting in brain slices.

Computational analysis: The lab has 30 PCs and 12 laptops for data and behavioral analysis, with an individual two-monitor PC for every lab member. Additionally, the lab has two high-powered analysis computers recently built for computationally intensive deep learning-based video analysis. These computers are each equipped with 64 GB of RAM, Intel Core i7-8700K CPUs and GeForce GTX 1080 Ti graphics processors selected specifically for compatibility with TensorFlow-based neural network applications. These computers are also configured with the Python package of the most recent release of DeepLabCut (2.0), which has been successfully used for behavioral tracking in the lab.

The research training plan in this application addresses high priority goals 1-2, 3 and 4 from the BRAIN 2025 Report.

Relevance to goal numbers 1-2:

Goal number two "Neuronal Dynamics: Recording Neuronal Activity Across Time and Space", emphasizes the importance of a) using novel approaches to map circuits, b) improving the capacity for large scale recordings of neural activity c) advancing recording technology.

For the mentored phase of my award I proposing to record medial prefrontal cortex (mPFC) and identify projectors to the lateral hypothalamus (LH) using ChR2-circuit-mapping in vivo using wireless technology to minimize the impact on the behavioral experiment. Moreover, I am proposing to use nVoke to manipulate mPFC input in the lateral hypothalamus with a red-shifted opsin while performing calcium imaging of genetically identified subpopulations. Both of these experiments employ techniques that are on the forefront of our field. Finally, for the independent phase of my award I will continue to do ChR2-circuit-mapping and in addition I am proposing an experiment in which I will do multi-site electrophysiology to understand the long-range connectivity between the hippocampus, mPFC and LH. Moreover, because most of these experiments are targeting specific subpopulations of neurons defined genetically (vGAT or vGLUT in LH) or by connectivity (between mPFC and LH or vHPC and mPFC), my experiments are also consistent with goal one which relates to increasing our understanding of the role different subpopulation of neurons play and mapping connectivity.

Relevance to goal number 3:

Goal number three "Manipulating circuit Activity" emphasizes the importance of establishing causal tests of hypotheses generated by observing neural activity. I am proposing to use optogenetics, both with ChR2, red-shifted excitatory opsin, and halorhodopsin to test specific hypotheses related to social dominance behavior and the importance of two circuits: the mPFC-LH circuit and the hippocampal-mPFC circuit. In addition, I will combine optogenetics and calcium imaging of genetically defined subpopulations to test a hypothesis of how mPFC input causally impacts activity of LH subpopulations during social dominance behavior.

Relevance to goal number 4:

Goal number four "The Importance of Behavior" the importance of having a more detailed understanding of behavioral dynamics. The BRAIN report emphasizes the specific importance of using freely-moving assays, machine learning and miniaturized devices to dramatically increase our understanding of how neuronal activity underlies behavior. I am proposing to use supervised machine learning to track the behavior of multiple mice while they interact in a novel social competition assay. Using Deep Learning tools, I am capable of tracking behavior of the same mice over time (hours/days). In addition, I will use unsupervised machine learning to create a profile of the behavioral motifs that are occurring during the social competition and how they relate to social rank. All of these efforts of quantifying behavior are to achieve the goal of understanding how activity in mPFC is linked to social dominance behavior and how mPFC circuits relate to social dominance behavior.

In summary, the proposed research and training plan are highly overlapping with multiple goals of the BRAIN Initiative.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Padilla Coreano, Nancy

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

POSITION TITLE: Posdoctoral Fellow

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Puerto Rico, San Juan, PR	BS	08/2011	Molecular Biology
Columbia University , New York, NY	MA	05/2013	Neurobiology and Behavior
Columbia University, New York, NY	PhD	08/2016	Neurobiology and Behavior
Massachusetts Institute of Technology, Cambridge, MA	Postdoctoral Fellow	09/2016-03/2019	Neuroscience
The Salk Institute, La Jolla, CA	Postdoctoral Fellow	04/2019-present	

A. Personal Statement

While in high school one of my passions was music, and I developed many questions about the relationship between music and the brain. How can music evoke emotions and change behavior was one of my many questions. This led me to seek opportunities in neuroscience. As a college student in the University of Puerto Rico, I took all available courses in neuroscience, including graduate courses, and found a neuroscience lab to join. Dr. Gregory Quirk accepted me into his lab and set the foundations for my research career. In the Quirk lab, I studied the neural circuits of learned fear and extinction in rats using pharmacology. My work in the Quirk lab led to a publication as a first author in which I demonstrated a novel time-dependent role for the midline thalamus in fear learning (Padilla-Coreano et al, 2012). Once I had this first research experience in the Quirk lab, I made a series of important realizations. The first one was that I could be a neuroscientist, I never knew a scientist until I met Greg, and thus it didn't seem feasible. The next realization was that I was meant to be a scientist. Solving the puzzle of an unknown research question fascinates me, especially when that question increases our understanding of the neurobiology of behavior<u>. From there on, I decided that my goal was to become a principal investigator and have my own laboratory, such that I can increase our knowledge about how the brain regulates behavior while mentoring the next generation of diverse neuroscientists.</u> My research experience in the Quirk lab led me to be interested in the neural circuitry of anxiety behavior. In graduate school, I joined the laboratory of Dr. Joshua Gordon at Columbia University. There, I combined projection-specific optogenetics and multi-site neurophysiology in mice to demonstrate pathway- and frequency- specific effects of the ventral hippocampal input to the medial prefrontal cortex (vHPC-mPFC) during innate anxiety. I found that the vHPC-mPFC pathway is required for anxiety-like behavior in mice. Moreover, that anxiety-induced increases in vHPC-mPFC (4-12 Hz) theta synchrony and spatial representations of aversion in the mPFC are dependent on direct vHPC input. This work was published in the journal *Neuron* (Padilla-Coreano et al., 2016).

In September 2016 I joined the lab of Dr. Kay Tye at MIT and in April 2019 I helped move the Tye lab to the Salk Institute which was an amazing opportunity to learn the practical aspects of setting up a lab. My postdoctoral work has consisted of two projects so far. The first project was a collaboration between Dr. Gordon, Dr. Christoph Kellendonk and the Tye lab to probe the causal relationship between theta oscillations in the vHPC-mPFC pathway and anxiety-like behavior. This work was published in 2019 in the journal *Neuron* (Padilla-Coreano et al., 2019). Completing this work was a great experience on how to successfully lead a collaboration between three laboratories. In addition, my main postdoctoral project is to understand the mPFC's role in social dominance and to identify circuits that modulate dominance. **My ultimate goal is to elucidate the neural circuits underlying social dominance behaviors and how they may differ for**

females and males. To this end, I designed an assay to measure dominance behavior in mice during a social competition assay. Using wireless electrophysiology and optogenetics I am studying how the mPFC output to the lateral hypothalamus contributes to social dominance. The skills and knowledge that I have gained so far form the foundation of my future research career, however, there are three major training goals that I must complete before being in a position to achieve my overarching career goals: 1) learn to use machine learning and statistical approaches to quantify behavior 2) expand my repertoire of circuit dissection techniques by learning in *in vivo* calcium imaging 3) increase my intellectual development to develop a unique framework on the circuits of social neuroscience. **I believe that achieving these career goals will ensure my successful transition to a tenure track faculty position in an R1 research university.** The proposed research and training plan in this K99/R00 application constitute an ambitious yet achievable plan to meet my career and training goals under the guidance of my mentor Dr. Kay Tye and my advisory team (Drs. Terry Sejnowski and Ed Callaway), and collaborator (Dr. James Curley).

Published work:

1. Padilla-Coreano N, Bolkan SS, Pierce GM, Blackman DR, Hardin WD, Garcia-Garcia AL, Spellman TJ, Gordon JA. Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron. 2016 Feb 17;89(4):857-66. PubMed PMID: 26853301; PubMed Central PMCID: PMC4760847.
2. Padilla-Coreano N, Do-Monte FH, Quirk GJ. A time-dependent role of midline thalamic nuclei in the retrieval of fear memory. Neuropharmacology. 2012 Jan;62(1):457-63. PubMed PMID: 21903111; PubMed Central PMCID: PMC3195904.
3. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. 2011 Jan;36(2):529-38. PubMed PMID: 20962768; PubMed Central PMCID: PMC3005957.

B. Positions and Honors

Professional Memberships

2009-present	Member, Society for Neuroscience
2016	Member of the Trainee Advisory Committee for the Society for Neuroscience

Honors and Fellowships

2008	College of Natural Sciences Dean's list, University of Puerto Rico
2010, 2014	Travel Award for University of Wisconsin (UW) Health and Emotions Conference, UW
2011	Neuroscience Scholar Program (NSP) fellowship recipient, **Society for Neuroscience**
2013	National Science Foundation Graduate Fellowship, **National Science Foundation**
2013	Ford Foundation Predoctoral Fellowship recipient, **Ford Foundation**
2015	Carl Storm Minority Fellowship Travel Award, Amygdala Gordon Conference
2016	Travel Award for the American College of Neuropsychopharmacology (ACNP) Meeting, ACNP
2017	Simons Center for the Social Brain Postdoctoral Fellowship recipient, **Simons Foundation**
2017	Career Development Travel Award, **Society for Neuroscience**
2019	Ford Foundation Postdoctoral Fellowship recipient, **Ford Foundation**
2019	Postdoctoral Enrichment Program fellow, **Burroughs Wellcome Fund Foundation**

Invited talks

2011	"Circuitos neurales del miedo" Centro de Restauración Neurológica (CIREN), La Habana, Cuba
2015	"The role of the direct hippocampal-prefrontal input in anxiety" Neuroscience Department Seminar at Columbia University, New York, USA
2016	"The role of the direct hippocampal-prefrontal input in anxiety" Winter Conference on Brain Research (WCBR), Colorado, USA

2019 "Studying the neural circuits underlying social dominance in mice" Simons Center for the Social Brain at Massachusetts Institute of Technology

C. Contribution to Science

1. Early career: As an undergraduate, I contributed to work dissociating the role of the prelimbic and infralimbic cortices in extinction learning and retrieval using rats and pharmacology. Moreover, I led a research project that provided evidence that the dorsal medial thalamus (dMT) has a time-dependent role in the retrieval of a fear memory. Moreover, we showed evidence suggesting that within the dMT the paraventricular thalamic nucleus was recruited into the fear circuit 24 hours after fear learning, rather than early after fear learning. This was the first demonstration of a time-dependent role of the thalamus in fear behaviors.

    a. **Padilla-Coreano N**, Do-Monte FH, Quirk GJ. A time-dependent role of midline thalamic nuclei in the retrieval of fear memory. Neuropharmacology. 2012 Jan;62(1):457-63. PubMed PMID: 21903111; PubMed Central PMCID: PMC3195904.
    b. Sierra-Mercado D, **Padilla-Coreano N**, Quirk GJ. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. 2011 Jan;36(2):529-38. PubMed PMID: 20962768; PubMed Central PMCID: PMC3005957.

2. Graduate career: I combined optogenetic inhibition and in vivo multi-site neurophysiology in mice to demonstrate pathway- and frequency- specific effects of the ventral hippocampal input to the medial prefrontal cortex (vHPC-mPFC) during innate anxiety. I found that the vHPC-mPFC pathway is required for normal anxiety behavior. Moreover, that anxiety-related increases in vHPC-mPFC (4-12 Hz) theta synchrony and spatial representations of aversion in the mPFC are dependent on direct vHPC input. Moreover, I participated in several collaborations that in which I contributed to two the projects related to the function of mPFC, one studying how parvalbumin interneurons in mPFC are affected by maternal immune activation and the second demonstrating the first in vivo awake recording of chandelier interneurons in mPFC.

    a. **Padilla-Coreano N**, Bolkan SS, Pierce GM, Blackman DR, Hardin WD, Garcia-Garcia AL, Spellman TJ, Gordon JA. Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron. 2016 Feb 17;89(4):857-66. PubMed PMID: 26853301; PubMed Central PMCID: PMC4760847.

    b. Canetta S, Bolkan S, **Padilla-Coreano N**, Song LJ, Sahn R, Harrison NL, Gordon JA, Brown A, Kellendonk C. Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons. Mol Psychiatry. 2016 Jul;21(7):956-68. PubMed PMID: 26830140; PubMed Central PMCID: PMC4914410.
    c. Lu J, Tucciarone J, **Padilla-Coreano N**, He M, Gordon JA, Huang ZJ. Selective inhibitory control of pyramidal neuron ensembles and cortical subnetworks by chandelier cells. Nat Neurosci. 2017 Oct; 20(10): 1377-1388; PubMed Central PMCID: PMC5614838

3. Postdoctoral career: So far during my postdoctoral career I have followed up on some of my findings from graduate school together with my former PI and current postdoctoral mentor. Using optogenetics, in vivo and in vitro electrophysiology, I demonstrated that oscillatory optogenetic stimulation at 8 Hz, but not other frequencies (2 Hz or 20 Hz), was sufficient to increase avoidance behavior and to facilitate neurotransmission between vHPC and mPFC. Thus, demonstrating that theta frequencies play a role in facilitating communication during anxiety states in the vHPC-mPFC pathway. In addition, while I work on my main postdoctoral project in the Tye lab, I collaborated with other Tye lab members to understand how distinct mPFC outputs modulate reward and aversion, and how ventral tegmental input modulates mPFC signaling during aversion.

    a. **Nancy Padilla-Coreano**, Sarah Canetta, Rachel M. Mikofsky, Emily Always, Johannes Passecker, Maxym V. Myroshnychenko, Alvaro L. Garcia-Garcia, Richard Warren, Eric Teboul,

Dakota R. Blackman, Mitchell P. Morton, Sofiya Hupalo, Kay M. Tye, Christoph Kellendonk, David A. Kupferschmidt, Joshua A. Gordon. Hippocampal-prefrontal theta transmission regulates avoidance behavior. *Neuron.* 2019 (in press).

b. C.M. Vander Weele, C.A. Siciliano, G.A. Matthews, P Nambury, E.M. Izadmehr, I.C. Espinel, E.H. Nieh, E.H.S. Schut, **N. Padilla-Coreano**, A. Burgos-Robles, C. Chang, E. Kimchi, A. Beyeler, R. Wichmann, C.P. Wildes, K.M. Tye. (2018) Dopamine enhances signap-to-noise ratio in cortical-brainstem encoding of aversive stimuli. Nature. PMCID: PMC6645392

Complete List of Published Work in My Bibliography: http://bit.ly/29dOEWI

D. Scholastic Performance

Class	Grade
University of Puerto Rico- Undergraduate courses (2007-2011)*	
Biology I	A
Biology II	A
Ecology	A
Molecular and Cellular Biology	A
Biochemistry	A
Neuropsychopharmacology	A
Neurobiology	A
Neurophysiology**	A
General Chemistry I	A
General Chemistry II	A
Organic Chemistry I	A
Organic Chemistry II	A
Columbia University-Graduate courses (2011-2016)	
Survey of Neuroscience	A-
Issues in Neural Circuitry	A-
Experimental Approaches in Neural Sciences	A
Communicating Science	A
Practicum in Teaching Psychology	A
Statistics for Basic Sciences	A
Biology of Neurological and Psychiatric Disorders	B+
Quantitative Approaches for Neuroscience***	P

*At the University of Puerto Rico grading system goes from A-F, no plus or minus grades.

**This was a graduate course taken as an undergraduate

***Pass/Fail class

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Nancy		Padilla Coreano	Ph.D.	PD/PI	75,000.00	12			75,000.00	14,250.00	89,250.00

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **89,250.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**89,250.00**

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

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period:** 1

C. Equipment Description

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

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

Additional Equipment: File Name:

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

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

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

Tracking Number: GRANT12955493

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period:** 1

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

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**114,250.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	114,250.00	9,140.00
		Total Indirect Costs	**9,140.00**

Cognizant Federal Agency Dept. of Health and Human Services, Jeanette Lu, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**123,390.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	123,390.00

L. Budget Justification* File Name:
sf_19_BUDGET_JUSTIFICATION1011499176.pdf
(Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Nancy		Padilla Coreano	Ph.D.	PD/PI	75,000.00	12			75,000.00	14,250.00	89,250.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **89,250.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) **89,250.00**

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

Tracking Number: GRANT12955493

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period:** 2

C. Equipment Description

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

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

Additional Equipment: File Name:

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

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

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

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2021 **End Date*:** 06-30-2022 **Budget Period:** 2

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

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**114,250.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	114,250.00	9,140.00
		Total Indirect Costs	**9,140.00**

Cognizant Federal Agency Dept. of Health and Human Services, Jeanette Lu, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**123,390.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	123,390.00

L. Budget Justification* File Name:
sf_19_BUDGET_JUSTIFICATION1011499176.pdf
(Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Nancy		Padilla Coreano	Ph.D.	PD/PI	0.00	12			0.00	0.00	0.00

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

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

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period:** 3

C. Equipment Description

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

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

Additional Equipment: File Name:

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

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

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

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2022 **End Date*:** 06-30-2023 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Independent Phase	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

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

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Dept. of Health and Human Services, Jeanette Lu, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification*
File Name:
sf_19_BUDGET_JUSTIFICATION1011499176.pdf
(Only attach one file.)

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

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Nancy		Padilla Coreano	Ph.D.	PD/PI	0.00	12			0.00	0.00	0.00

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

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

B. Other Personnel

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

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

Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period:** 4

C. Equipment Description

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

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

Additional Equipment: File Name:

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

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

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

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

ORGANIZATIONAL DUNS*: 078731668
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2023 **End Date*:** 06-30-2024 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Independent Phase	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

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

Cognizant Federal Agency Dept. of Health and Human Services, Jeanette Lu, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification* File Name:
sf_19_BUDGET_JUSTIFICATION1011499176.pdf
(Only attach one file.)

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

Tracking Number: GRANT12955493
Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2024 **End Date*:** 06-30-2025 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Nancy		Padilla Coreano	Ph.D.	PD/PI	0.00	12			0.00	0.00	0.00

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

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

B. Other Personnel

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

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

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2024 **End Date*:** 06-30-2025 **Budget Period:** 5

C. Equipment Description

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

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

Additional Equipment: File Name:

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

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

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

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

ORGANIZATIONAL DUNS*: 078731668

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Salk Institute for Biological Studies

Start Date*: 07-01-2024 **End Date*:** 06-30-2025 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Independent Phasef	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

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

Cognizant Federal Agency Dept. of Health and Human Services, Jeanette Lu, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification* File Name:
sf_19_BUDGET_JUSTIFICATION1011499176.pdf
(Only attach one file.)

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

Tracking Number: GRANT12955493 Funding Opportunity Number: PAR-18-814 . Received Date: 2019-10-15T20:17:09.000-04:00

BUDGET JUSTIFICATION

Mentored Phase (K99) - Years 1-2

Personnel

Nancy Padilla Coreano, Ph.D., Principal Investigator, (effort = 12 calendar months) will be responsible for all experiments, analysis, manuscript preparation and publication, and will present work from this study at scientific conferences and seminars.

Research Support

Materials and Supplies $10,500/year: Materials used in the proposed optogenetics and electrophysiology experiments, including electrode connectors, electrode-grade insulated wire, optic fibers implants, patch cords, circuit boards and other electronic components. In addition, a computer with a GPU will is needed in order to perform deep learning analyses.

Animals $7,000/year: Purchase and housing of transgenic mice for the proposed research project. Estimated costs are based on per diem fees at the Salk Institute's Animal Resources Department and the number of experimental mice used in experiments.

Travel $3,500/year: Dr. Padilla’s travel to scientific conferences to present her research (See ‘Career Development and Training Activities’ section of the Candidate Information document).

Publication and Presentation Fees $4,000/year: Fees associated with publication of the project, as well as poster printing and any other non-travel expenditures required for presentation of the research at conferences.

Independent Phase (R00) - Years 3-5

As outlined in the funding opportunity announcement guidelines, a detailed budget for each year will be prepared upon activation of the R00 phase of this award.

Personnel

Nancy Padilla Coreano, Ph.D., Principal Investigator, (effort = 9 calendar months) will request 75% support to allow for any teaching responsibilities or administrative duties in this new position, and for commitment to other pilot experiments or grants from other funding sources if needed.

Research Technician, (effort = 12 calendar months). Salary support will be requested for a full-time research technician to maintain animal colonies and assist with behavioral experiments for the R00 phase experiments.

Other Costs: Costs will include funds for research supplies, animals, travel costs, and publication fees similar on a per year basis to those outlined in the K99 phase, as well as additional research supplies for electrophysiology and optogenetic experiments such as viruses. Costs will vary based on animal housing costs at the institution where the R00 award is issued.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		178,500.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		178,500.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		797,000.00
1. Materials and Supplies	50,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	747,000.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		975,500.00
Section H, Indirect Costs		18,280.00
Section I, Total Direct and Indirect Costs (G + H)		993,780.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		993,780.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ❍ No

If "Yes" to euthanasia

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

● Yes ❍ No

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

2. *Program Income Section

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

❍ Yes ● No

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

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

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3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ❍ No

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

*Previously Reported: ❍ Yes ❍ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Section / Item	Attachment
Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Candidate Section	
2. Candidate Information and Goals for Career Development	07_Candidate_Information1011499134.pdf
Research Plan Section	
3. Specific Aims	08_SpecificAimsnewest1011499019.pdf
4. Research Strategy*	09_Research_Strategy1011499149.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	10__RESPONSIBLE_CONDUCT1011499021.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Statements_by_Mentors1011499135.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Letters_of_Support_combined1011498946.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	ENVIRONMENT1011499022.pdf
11. Institutional Commitment to Candidate's Research Career Development	Signed_Commitment_Letter1011498991.pdf
Other Research Plan Section	
12. Vertebrate Animals	15_NIH_Vertebrate_Animals1011499146.pdf
13. Select Agent Research	16_SELECT_AGENT_RESEARCH1011499024.pdf
14. Consortium/Contractual Arrangements	
15. Resource Sharing	17_RESOURCE_SHARING1011499136.pdf
16. Authentication of Key Biological and/or Chemical Resources	
Appendix	
17. Appendix	

PHS 398 Career Development Award Supplemental Form

Citizenship*:

18. U.S. Citizen or Non-Citizen National?* ● Yes ❍ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

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: ❑

1. CANDIDATE BACKGROUND

As a neuroscientist, I seek to elucidate how the brain executes behaviors that come naturally to us, such as avoiding danger, seeking pleasure or socially interacting. My scientific training has prepared me to investigate the neurobiological basis of innate behaviors in animal models. I started college with a strong interest in understanding the neural basis of behaviors. In Dr. Gregory Quirk's Lab, I learned the basics of using behavioral paradigms to study fear in rats. Specifically, I used fear conditioning paradigms, wherein a tone is associated with a shock, and neuropharmacology to further our understanding of how the amygdala, the medial prefrontal cortex (mPFC) and the mediodorsal thalamus contribute to fear learning and expression. This work resulted in two peer-reviewed articles[1,2], one of which has over 700 citations[2]. Besides learning the fundamentals of behavioral neuroscience, I also learned that not all emotions all learned. This realization drove me to study the neural basis of avoidance behavior, an innate behavior that comes naturally to all animals.

For my doctoral thesis, I joined the laboratory of Dr. Joshua Gordon at Columbia University. There, I studied how the ventral hippocampal input to the mPFC (vHPC-mPFC) encodes anxiety-like behavior in mice. I performed simultaneous recordings in the vHPC and the mPFC, and used optogenetics to manipulate activity in this pathway during behavior. My work demonstrated that optogenetically inhibiting the vHPC-mPFC circuit disrupted both anxiety-like behavior and the mPFC neural representations of aversion. Moreover, vHPC-mPFC inhibition reduced theta (4-12 Hz) synchrony between vHPC and mPFC, in a pathway-, frequency- and task-specific manner. These results revealed a specific role for the vHPC-mPFC projection in anxiety-related behavior and its neural representation. This work was published in 2016 in *Neuron*[3]. Next, I investigated whether optogenetic stimulation of the vHPC-mPFC at a theta frequency, 8 Hz, was sufficient to increase avoidance behavior. I optogenetically activated vHPC terminals in the mPFC using an oscillatory light stimulus, to mimic the synchronous oscillations observed during anxiety in this pathway. Oscillatory terminal activation at 8 Hz was sufficient to increase avoidance behavior. Using in vivo and ex vivo electrophysiology we demonstrated that 8 Hz terminal activation enhanced vHPC-mPFC neurotransmission more than other frequencies and stimuli patterns. Finally, 8 Hz oscillatory stimulation of vHPC terminals entrained neural activity in the entire vHPC-mPFC network during exposure to an anxiogenic environment. In collaboration with my postdoctoral advisor, Dr. Kay Tye, this study and published in *Neuron* this year[4]. These findings, combined with my prior results collectively demonstrate bidirectional effects of optically manipulating the vHPC-mPFC circuit, and point to a privileged role for theta-frequency activity in sustaining information transfer within this circuit.

In addition to my work in the vHPC-mPFC circuit, I collaborated with several groups to contribute to our understanding of mPFC microcircuitry. Together with Dr. Christoph Kellendonk's group, I used *in vivo* electrophysiology and optogenetics to study how mPFC parvalbumin interneurons contribute to anxiety-like behavior and working memory deficits seen in adult mice with prenatal immune activation[5]. Furthermore, in collaboration with Dr. Joshua Huang we targeted and characterized the poorly studied chandelier cells. In 2017, we published that these cells have precise microcircuitry in the mPFC by innervating preferentially pyramidal cells that project to the amygdala. Second, there has been a long debate on whether chandelier cells are in fact inhibit or excite pyramidal cells, and I provided the first *in vivo* evidence that they are inhibitory *in vivo*[6].

In September 2016, I started my postdoctoral training in the laboratory of Dr. Kay Tye at the Massachusetts Institute of Technology. Earlier this year, I had the opportunity to help Dr. Tye move the lab to the Salk Institute. This unique opportunity exposed me to the process of setting up a lab, an invaluable experience for my future. As a postdoc, I have been investigating how the brain encodes social hierarchies. My goal is to elucidate how the medial prefrontal cortex modulates social rank via downstream hypothalamic nuclei. My previous work studying the mPFC in the context behavior, and its micro and macro-circuitry gives me an excellent scientific framework. For my independent work, I plan to combined my previous training in multi-site electrophysiology with the new skills I will obtain during the K99 phase to study how long-range circuits encode social dominance in females and males. **In order to complete my research plan I will need to fill certain gaps still present in both my intellectual as well as practical skillsets. This application is designed to provide me the mentorship and training experiences I need to achieve my career goals. Furthermore, those new skillsets and training will set me up for a successful career as an independent researcher.**

2. CAREER GOALS AND OBJECTIVES

Career Goals: My overarching career goal is to obtain a tenure-track position at a leading, research-based academic institution. Specifically, I want to contribute to the field by expanding our understanding of the neural circuits and computations governing social behaviors, including dominance and social memory, and how these processes are disrupted in mouse models for psychiatric disorders. Beginning with my undergraduate studies and continuing through my time in graduate school, I have been interested in the neurobiological interface between the brain and behavior. I plan to investigate the neural mechanisms controlling social dominance using complex behavioral assays, *in vivo* recordings and by probing circuit function at different spatial and time scales.

Distinction from mentor's research: The long-term goal of my research is to identify the circuits that encode and modulate social behaviors, **specifically behaviors associated with social competition in both female and males**. I am mindful, as I start my independent research career, that I will face the challenge of establishing independence from Dr. Tye and her current and future trainees. I believe that the training in my K99 will provide me with the right tools to achieve my goal. First, my training in multi-site electrophysiology to quantify synchrony and functional connectivity of long-range circuits is well outside the scope of study of the Tye lab. Second, Dr. Tye's research relating to social behavior is focused on studying social isolation and social homeostasis, and is not focused on comparing female and male animals. Moreover, my line of research differs from my doctoral mentor Dr. Josh Gordon, whose research focuses on the neural circuit related to working memory dysfunction in mouse models of genetic susceptibility to schizophrenia and related disorders.

In order to achieve my overarching goal, my mentors and I have identified three specific areas that will need to be further developed to allow me to perform independent, high impact research. I will describe each of these goals below, and throughout this application, and explain how this award will provide training necessary to reach independence. Importantly, I believe that the K99/R00 award mechanism is an ideal way for me to complete my career goals. This award will result in substantial career development opportunities that will not only provide necessary professional skills to becoming a faculty member, but also facilitate my expansion into an entirely new area of research with expertise, technical proficiency, and collaborations. My five-year proposal includes two years of additional supervised/postdoctoral training to learn quantitative and statistical analyses for behavior, in vivo calcium imaging and circuit tracing techniques, as well as equip me with the skills and experience to lead an effective research team. ***This award is thus an essential step in my training and will allow me to reach independence as a researcher, and ultimately will put me in an excellent position to become a leader in the field of social neuroscience.***

Summary of gaps and training goals: During the two additional years in the lab I will work on the following specific goals

A. Develop expertise in statistical approaches for behavioral analysis: Our understanding of how the brain encodes social behaviors relies on how good we can quantify these behaviors. Therefore, developing skills to quantify behavior is the highest priority of my remaining postdoctoral training. Although I did behavioral experiments in mice during graduate school, I never performed complex analysis on the behavior. Thus, this is a training gap I will close with this career development plan. During graduate school I took a course on statistics and had heavy experience programming with *Matlab,* but I did not receive any formal training on machine learning, which is central for doing detailed behavioral analyses proposed on my research. Towards this goal I will take two courses, one on programming with Python and the other on Machine Learning, and I will benefit from the guidance of Drs. Terry Sejnowski and James Curley. The formal coursework and guidance from my mentors will allow me to meet my goal of learning to effectively apply existing machine learning tools for behavioral analysis. In addition, through my collaboration with Dr. Curley I will learn to select the correct statistical tests for the behavioral metrics I will be quantifying.

B. Expand repertoire of *in vivo* techniques to interrogate neural circuits: I already have expertise using in vivo electrophysiology, both single units and local field potentials, to measure functional connectivity during behavior. This expertise will serve me well for the proposed research and my independent phase, and I am fully capable of setting up in vivo electrophysiology in my future laboratory. However, I lack knowledge of other techniques to probe neural circuits, especially anatomical tracing and neural recording methods that allow longer timescales than electrophysiology. I will use my training time to close this gap by learning in vivo 1-Photon calcium imaging and viral-tracing for circuit mapping, both techniques that are already successfully established in the Tye Lab.

C. Improve leadership and management skills: In order to be a successful lab head, I need to master leadership and management skills. I have been supervising students in the Tye lab for a few years, but I need more time to improve my skills as a leader and to develop a leadership style that works for me. By supervising graduate students, visiting scholars and technicians, I will learn useful mentoring and management skills that will serve me in my own laboratory.

D. Scholarly development: In order to be recognized in my field and successful in the job market I must network and present my work thoroughly to different audiences. By attending and presenting my research at national and international conferences, as well as attending and presenting in the laboratories of my advisors and collaborators, I will strengthen my communication skills and conceptual knowledge of my field.

3. CAREER DEVELOPMENT AND TRAINING ACTIVITIES

Below I detail different aspects of the development and training plan. First, I detail the mentoring team, and then I explain the activities that will help me close the training gaps and meet my training objectives. In addition, we have designed specific benchmarks to facilitate my transition into an independent faculty position such as demonstrating mastery of key techniques, publishing, and networking with chairs of neuroscience departments and mock job talks and chalk talks.

A. Mentors and Collaborators: Kay M. Tye, PhD (Primary Mentor): Dr. Tye is my primary mentor and as such will take the lead role in my scientific and professional development. Dr. Tye is emerging as a world leader in systems neuroscience, and is an expert in all of the techniques proposed in this application. She is truly invested and passionate about the development of her trainees, a fact that is supported by numerous mentoring awards she has received. She has now helped four past postdoctoral trainees find tenure-track positions. Dr. Tye and I will discuss my progress at our standing weekly meetings. These meetings have been integral to my success thus far and have provided an opportunity to discuss career development and research strategies. For example, with Dr. Tye's help and advice I have already been awarded multiple prestigious fellowships (e.g. Simons and Ford Foundations). Additionally, in the Tye Lab meetings I have still more opportunities to present my data, and get feedback, from Dr. Tye and lab members.

I believe that Dr. Tye is an ideal mentor for me, however we do acknowledge that she has had a relatively short history of transitioning postdoctoral trainees to independence. My advisory team is well-known for their long history of mentorship. Together, my mentor and advisory committee members have advised and guided hundreds of students and postdocs through their labs, with an impressive history of obtaining their trainees tenure-track positions. Together, this committee represents researchers from differing career stages, junior to senior, with differing, but all highly successful, scientific and leadership styles.

1. **Dr. Terry Sejnowski** is a world-renowned expert on Neural Networks and Computational Neuroscience, and his seminal work on Neural Networks and deep learning changed the field. His laboratory uses both experimental and modeling techniques to study the biophysical properties of synapses and neurons and the population dynamics of large networks of neurons as well as to understand behavior. He has helped nearly 100 postdoctoral trainees transition to independence. Dr. Sejnowski will have one-on-one meetings with me to discuss my progress on applying machine learning for social behavior analysis. Moreover, I will attend the lab meetings of his group to improve my conceptual understanding of the applications of machine learning for neuroscience. Dr. Sejnowski will be part of my advisory committee.
2. **Dr. Edward Callaway** is a professor in the Systems Neurobiology laboratory at the Salk Institute, and the Vincent J. Coates Chair in Molecular Neurobiology. During the initial period of my K99 training, Dr. Callaway and I will meet every second month to discuss research progress, and then meet more frequently as I approach my transition to independence. Dr. Callaway has an outstanding record of both excellence in neuroscience research and helping trainees secure tenure-track positions, and he will provide me with training and guidance to prepare for my academic job search, as well as navigating publishing and securing funding as an independent investigator. Dr. Callaway will be part of my advisory committee.
3. **Dr. James Curley** is an expert on social dominance behaviors in mouse models. His research has served as foundation for the field that studies the neural circuits of social dominance. In addition, he is an expert in applying statistics to analyze complex social behaviors in mice. Dr. Curley will serve to enrich my conceptual knowledge of social behaviors in female and male mice and to advise on the statistical analysis and data interpretation for my K99 phase. Dr. Curley will serve as a collaborator for this application.

B. Mentor Phase Training and Objectives

Develop expertise in applying statistical and computational approaches for behavioral analysis: My top goal for the K99 phase of this award will be to develop expertise in the use of machine learning approaches to automatically track and classify behavior, and to do social network analysis of behavior. I will start by learning to use DeepLabCut, a recently developed deep learning neural network, to track body position during social behaviors. To this end I will take two courses at UCSD (COGS 118A and COGS 18) which will provide formal instruction in Python and machine learning approaches. Under the guidance of Dr. Terry Sejnowski, I will use unsupervised clustering of the tracked body positions to cluster the behavioral data into distinct behavioral motifs (Aim 1). Next, in the collaboration with Dr. James Curley, I will learn to use social network analyses and other relevant statistical analyses to quantify social dominance differences, even if there are subtle changes in behavior, and how manipulating neural circuits changes behavior. I will have video calls monthly with my collaborator and monthly in person meetings with my advisors to ensure progress for this goal. Once a year I will visit the lab of my collaborator, Dr. Curley, to present my research progress and discuss challenges and approaches, and twice a year we will have in person meetings. Altogether learning these approaches will position me to become a successful independent investigator with the right tools to dissect the neural basis of social behaviors.

Expand repertoire of in vivo techniques to interrogate neural circuits: I already have expertise using in vivo electrophysiology, both single units and local field potentials, to measure functional connectivity during behavior. This expertise will serve me well for the proposed research and my independent phase, and I am fully capable of setting up in vivo electrophysiology in my future laboratory. However, considering the importance of studying subpopulations of cells across longer time periods to dissect neural circuits, I decided to learn single cell calcium epifluorescence imaging and rabies tracing to determine anatomical connectivity. Our lab has published several papers with single cell calcium imaging and Dr. Callaway will provide additional support for rabies tracing tools, so my mentor and advisor are well positioned to guide me as I learn these new techniques. Together, these new techniques will allow me to interrogate circuits at different scales, from anatomical connectivity to functional connectivity at different time scales.

Intellectual and scholar development through meetings and presentations: To develop independence it is important that I seek additional conceptual frameworks that will allow me to think creatively and create my own niche of research. To this end I will attend group meetings from my advisory committee members and I will attend conferences on social neuroscience and computational neuroscience. I believe that attending these meetings and conferences will allow me to best utilize the computational tools that are constantly being develop to achieve my research goal of dissecting the neural circuits of social behaviors at both the micro (single-cells) and macro-levels (ensembles and oscillations). By attending the lab meetings of Drs. Terry Sejnowski and Ed Callaway every other month, I will expand my expertise on computational approaches in Neuroscience and Circuit Dissection. Both of these laboratories are in the same building as the Tye lab, facilitating constant communication between all lab members and PIs. Dr. Sejnowski's group holds computational tea times every day at 3:30p where there are intellectual and technical discussions. I have started attending these tea times to discuss ongoing progress and plans with machine learning experts from the Sejnowski group, and will continue to do so. In addition, during the K99 phase, I will attend 3 international conferences per year that are relevant to my research and new skills I am acquiring. I will attend the Society for Social Neuroscience (S4SN) meeting and the Computational and Systems Neuroscience (COSYNE) meeting, and the American College of Neuropsychopharmacology (ACNP), which is known for being great for professional development and networking. Attending each of these conferences will provide development for different components of my training grant. At S4SN, I will get a chance to discuss the nuances of my research with the most relevant scientists considering my research, in addition my collaborator Dr. Curley attends this conference every year and we will meet during the conference. COSYNE will foster my computational and quantitative skillsets, as I will be able to discuss the quantitative approaches and caveats of each approach with experts on machine learning and other statistical approaches. Finally, ACNP will provide me a more translational window into my research and will serve to network as I transition to the job market.

Improve leadership and management skills: By leading a team of a graduate student, master student and technician and by receiving coaching from my primary mentor in our one-on-one meetings. These meetings with my mentor have already served me to acquire useful skills that I hope to apply and practice further during the K99 phase of this award. In addition, the Salk provides workshops on management and seminars that I will attend during the K99 phase of this award. Overall, I am confident that the useful mentoring and management skills I am learning from my mentor and by leading a team will serve me for my future laboratory and will contribute to my success as a PI.

C. Benchmarks for Success
Some benchmarks of success for my training plan will be 1) the successful publication of a first-author study based on the present research proposal, and 2) securing a tenure-track position at a leading research institution. Additional benchmarks will include publication of data using the circuit dissection and computational analysis approaches outlined above, and presenting my research at 2-3 international neuroscience meetings. To ensure I achieve these goals, I will follow the training plan and I will provide consistent progress updates to my mentor and committee, who will provide feedback and help me determine how to overcome challenges.

D. Transitioning to Independence
To be a successful independent scientist I will have to master not only the skills listed above, but also grant and manuscript writing. I will continue to practice my writing skills under the supervision of my mentor during the preparation of my manuscript and by preparing drafts of future grants I would submit during my independence phase. In addition, in the second year of my mentored phase I will give practice job talks and chalk talks in the Tye lab and my advisor's lab meetings. These practice talks will serve to prepare me intellectually and performance-wise for the type of questions that I might receive and to communicate with clarity.

Activity	Year 1 (K99)	Year 2 (K99)	Year 3 (R00)	Year 4 (R00)	Year 5 (R00)
Methods Training	Machine learning	Tracing and in vivo imaging			
Professional Development Activities	COSYN Workshops Present in - Sejnowski and Callaway meetings	Management leadership seminars Present in Sejnowski and Callaway meetings			
Coursework	UCSD COGS 118A (Machine and COGS (Python)	Responsibl conduct of Research			
Training Mentors and Collaborators	Monthly with Drs. and Monthly calls with Curley Biweekly with Dr. Tye	Monthly with Drs. and Monthly calls with Curley Biweekly with Dr. Tye	Meetings Senior Faculty mentor and colleague Monthly calls with committee.	Meetings with Senior Faculty mentor and junior colleague	Meetings Senior Faculty mentor and colleague
Research Goals	Aims 1 and 2	Aims 2 and 3	Aim 4.1	Aim 4.2	Aim 4.3
Manuscript and Writing	Submit 1	Submit 2 Submit NARSAD grant	Draft R01 grant	Submit R01 grant	Submit manuscript 3 last author)
Scientific Conferences	S4SN, and ACNP	S4SN, ACNP COSYN		S4SN and SfN	
Mentoring	Training student and technician	Training student and technician	Training student, undergrads technician	Training graduate student and postdoc	Training student and postdoc

SPECIFIC AIMS

How do we adjust our behavior according to the social context? Social rank provides that context for many animals. Adjusting behavior according to social rank is critical for survival, as it influences social interactions and access to resources. Animals with higher social rank express more dominance behaviors; that is, they express more agonistic behaviors and they win more often during social competition. Studies with humans, non-human primates and rodents have shown that the medial prefrontal cortex (mPFC) is implicated in social dominance. For instance, manipulating the mPFC in mice affects dominance expression by increasing winning during social competition. It has also been demonstrated that the ventral hippocampal (vHPC) input to mPFC is necessary for social memory, which may be critical for social dominance expression. Further, our lab has shown that manipulating distinct lateral hypothalamic (LH) subpopulations, gabaergic cells (vGAT) and glutamatergic cells (vGLUT), which are involved in energy and motivation, can modulate social investigation. **Yet, to date it remains unknown how the mPFC encodes social dominance and which downstream projections are recruited during social competition.** I propose to test a model in which the mPFC receives social memory information from the vHPC and guides behavioral output via modulation of vGAT and vGLUT in the lateral hypothalamus. Our preliminary data show that activation of mPFC cells that project to LH ($mPFC^{LH}$) increases dominance behaviors in mice. Since there are limited tools to characterize murine social behavior, as the existing social assays are simplistic and lack trial-structure, I developed a trial-based social competition assay in which mice compete against cagemates of known social ranks for a reward signaled by a tone. Moreover, I will use machine learning tools to better quantify behavioral differences which will facilitate identifying neural correlates of social rank. By combining these novel behavioral assays with circuit-level manipulations, wireless electrophysiology, and in vivo imaging, we can enhance our ability to dissect the neural circuits underlying social behaviors. **Using these approaches, I will test the hypothesis that the tripartite vHPC-mPFC-LH circuit encodes social dominance**.

Aim 1 (K99): Characterize the behavior of dominant and subordinate mice in a reward competition task. The goal of this aim is to use automatic tracking to build a detailed profile of how behavioral motifs differ across dominance in mice during the reward competition assay described above. Our *preliminary data* show that dominant mice, as defined by the tube test, win most of the rewards and spend more time at the reward port. Using DeepLabCut for mouse pose tracking, unsupervised machine learning and network analysis, I will characterize the behavioral motifs that occur during this competition assay. I *hypothesize* that dominant and subordinate mice have distinct frequencies and transition probabilities of behavioral motifs.

Aim 2 (K99): Test whether the mPFC-LH circuit encodes social dominance via vGLUT and vGAT LH subpopulations. 2.1 I will wirelessly record mPFC single-units and phototag $mPFC^{LH}$ projectors during the reward competition. *Preliminary data* show that mPFC and specifically $mPFC^{LH}$ cells encode winning and action of the competitor. I *hypothesize* that $mPFC^{LH}$ projectors will be modulated by winning trials as compared to losing or alone trials (no cagemate), and that distinct subpopulations will be active during competition with dominant vs subordinates. 2.2 In vGAT::Cre and vGLUT::Cre mice, I will image single-cell calcium activity of LH vGAT or vGLUT cells while stimulating the mPFC inputs with red-shift opsin to determine the functional downstream effect of mPFC-LH activation. I *hypothesize* that mPFC activation will modulate vGAT and vGLUT LH subpopulations differently depending on social rank, resulting in higher vGAT LH activity in dominant mice.

Aim 3 (K99): Examine whether the mPFC-LH activity modulates social dominance behavior. *Preliminary data* show that optogenetic excitation of $mPFC^{LH}$ projectors increases social dominance in the reward competition task without affecting general effort. Using optogenetics, DeepLabCut and unsupervised clustering, I will determine how $mPFC^{LH}$ activity affects behavior in the reward competition task. I *hypothesize* that activation of $mPFC^{LH}$ projectors will increase dominance behaviors while inhibition of $mPFC^{LH}$ projectors will decrease dominance behaviors.

Aim 4 (R00): **Test the hypothesis that social memory is upstream of social dominance**. The vHPC-mPFC circuit is necessary for social memory. Furthermore, the vHPC synchronizes with the mPFC at different frequencies depending on the behavioral state, thus providing a possible way to potentially encode dominance. By combining the quantitative approach to study social behavior and optical techniques from my mentored phase, with my expertise in using multi-site electrophysiology and local field potentials (LFPs), I will investigate the role of the vHPC in social dominance. Specifically, I will test the following hypotheses. *Hypothesis* 4.1 (H4.1) Unilateral inhibition of vHPC-mPFC input will result in changes in mPFC encoding of dominant, but will not change social behavior. H4.2 Bilateral inhibition of vHPC-mPFC will disrupt social dominance expression. H4.3 vHPC-mPFC inhibition will disrupt dominance-related LFP synchrony between mPFC and LH.

Altogether, this research will provide a new approach to study social dominance in mice and will increase our understanding of how the mPFC, its inputs and outputs modulate social behavior. Moreover, completion of this research will provide me with training in statistical approaches for behavioral analysis and circuit dissection tools required for me to become an expert in social neuroscience and to start an independent and successful research program.

RESEARCH STRATEGY

Significance

Social behaviors are central for survival and health[7–11]. However, little is known about how the brain controls them. Some psychiatric disorders are characterized by disrupted social behaviors[9,12,13]. Elucidating the neural circuits that underlie social behavior is important for understanding basic brain function, as well as to inform potential solutions for social behavior deficits seen in psychiatric disorders.

A basic tenet of animal social behavior is following social rank. In most social species, groups of animals self-organized into dominance hierarchies[14]. Humans, like many animals, form social hierarchies that allow them to take avoid conflict and take better advantage of the environment as a group[8]. Animals with higher social rank express more dominance; they get the majority of the resources and win most conflicts[15]. After cohabitation, mice quickly form strong and stable dominance hierarchies[16,17]. In mice, social rank dictates many aspects of behavior, such as susceptibility to stress and anxiety traits[18]. Considering these facts and the existence of tools for studying and dissecting neural circuits in mouse models, mice make an excellent model to study the neural circuits underlying social dominance.

Cross-species evidence points to the medial prefrontal cortex (mPFC) being involved in social dominance[14,19,20]. Across species dominance is associated with increased activity in the mPFC[16,21]. Furthermore, mPFC optogenetic activation in subordinate mice leads to increased winning during social competition[22]. Although there is strong evidence for mPFC's involvement in social dominance several questions remain unanswered. 1) What type of social information is encoded in the mPFC? 2) Which mPFC outputs modulate dominance behavior? 3) From where does mPFC receive social information?

The lateral hypothalamus (LH) drives motivated behaviors such as feeding[23,24] and social investigation[25], and receives a dense projection from the mPFC. My preliminary data suggests that the mPFC-LH pathway drives social dominance. Within the LH, activation of GABAergic cells (vGAT) increases motivated behaviors[24], while activation of glutamatergic LH (vGLUT) decreases motivated behaviors[23,25]. Differential recruitment of these subpopulations could provide a mechanism for mPFC to modulate motivation based on social rank. However, the mPFC functional connectivity to these LH subpopulations is unknown. How is the mPFC receiving social information to guide dominance behaviors via its outputs? One possibility is via the ventral hippocampus (vHPC). Multiple studies have shown that the vHPC is necessary for social memory[26,27]. More recently a study showed that activation or inhibition of vHPC cells that project to mPFC disrupt social memory in mice, as measured by the ability to distinguish novel from familiar mice. This suggests that vHPC provides mPFC with information about social identity[28]. Moreover, there is precedent for the vHPC input being necessary for mPFC's ability to encode spatial valence in an anxiogenic context and in a working memory assay[3,29]. Thus, I hypothesize that mPFC uses vHPC input to encode social dominance. Interestingly, the vHPC synchronizes with the mPFC at different frequencies depending on the behavioral states[4,29,30]. I hypothesize that social dominance could be reflected by differences in local field potential synchrony and frequency activity across the vHPC-mPFC-LH tripartite circuit.

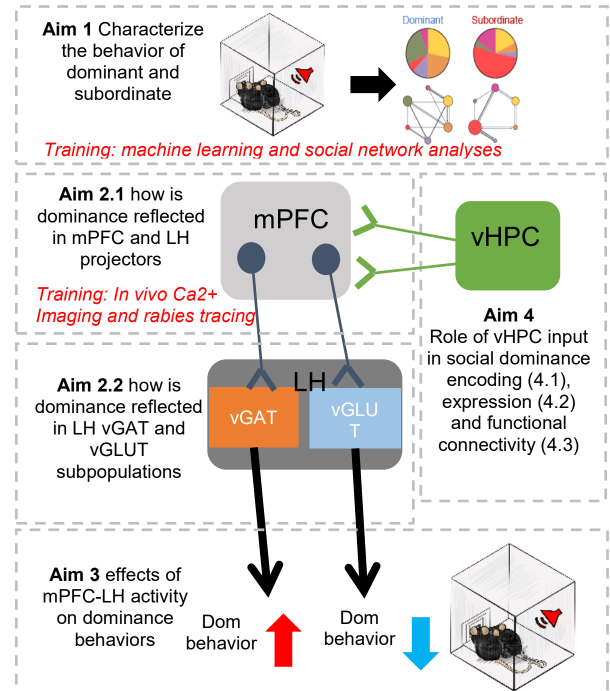

Figure 1: Diagram summarizing all aims for this grant and training goals for Aims 1 and 2.

Novel Assay: In order to tackle these questions, it is necessary to develop more quantitative measurements of social dominance behavior. Existing assays, such as the tube test or urine marking test are reliable but lack the structure and timescale that is beneficial for identifying neural correlates of social dominance. I have developed a trial-based reward competition assay that allows more quantitative measures of dominance behavior. This assay is ethologically relevant since dominant mice obtain most of the rewards relative to subordinates, mimicking the priority access dominants have to resources in the wild[15,31]. Using this assay, machine learning and statistical approaches to quantify behavior, I will describe how dominant and subordinate mice behave differently during competition (**Aim 1 K99**; Fig 1). This will facilitate the identification of mPFC neural correlates of behavior and changes in dominance behavior caused by circuit disruptions. Using in vivo electrophysiology, single-cell calcium imaging and tracing techniques, I will test the hypothesis that the mPFC-LH circuit encodes social dominance via vGLUT and vGAT LH subpopulations **(Aim 2 K99**; Fig 1**).** Our preliminary data show that optogenetic activation of mPFC cells that project to LH ($mPFC^{LH}$) leads to increased dominance behaviors

during social competition. Thus, I will next test the hypothesis that mPFC-LH activity modulates social dominance behavior expression (**Aim 3 K99**; Fig 1). As I transition to my independent phase, I will combine my previous training and K99 training to test the hypothesis that social memory is upstream of social dominance. Specifically, I will test the hypothesis that vHPC input is required for mPFC encoding and expression of social dominance. Finally, I will test the hypothesis that vHPC is required for functional connectivity between mPFC and LH during social dominance (**Aim 4 R00**; Fig 1).

Overall, I propose to test a model in which the mPFC receives social information from the ventral hippocampus (vHPC) and guides behavioral output via hypothalamic projections. **The overall hypothesis of this grant is that the tripartite vHPC-mPFC-lateral hypothalamic circuit encodes social dominance.** While this research plan is limited to 5 years, the experiments and training will provide ample opportunity to expand on findings (e.g. potential sex differences) and to apply similar approaches to probe the role of other mPFC outputs in social dominance.

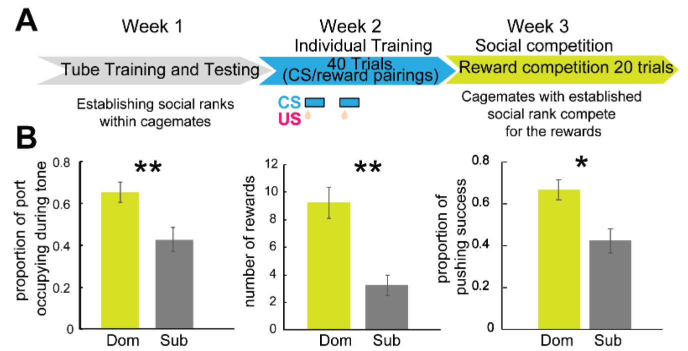

Figure 2: Dominant male mice win the Reward Competition. A. Schematic of behavioral protocol. B. During reward competition the dominant (Dom) mouse, as defined by tube test, occupied the reward port (left), obtained more rewards (center) and succeeded displacing competitor when pushing (right) more than subordinate (sub). Data from 12 pairs of cagemates collected by N.P.C. *p<0.05, **p<.01 ttest.

Innovation

1) Aim 1 uses novel machine learning approaches to build a detailed profile of how behavioral motifs differ across dominance in mice, which has never been done before.
2) Despite previous studies implicating the mPFC in social dominance, no study to date to my knowledge has investigated the output subpopulations of mPFC that are involved in social dominance.
3) Aim 2 will utilize cutting-edge techniques to map the mPFC-LH circuit's role in social dominance at multiple levels: ChR2-mediated phototagging[32] to characterize what the circuit encodes during behavior, rabies tracing to characterize anatomical connectivity, and in vivo imaging in LH subpopulations to determine how they are modulated by mPFC input.
4) The use of wireless electrophysiology during a freely moving trial-based social competition assay will facilitate identifying behavioral motifs of dominance that are limited by tethering mice with bulk electrophysiology tethers. - Moreover, the use of this assay will facilitate identifying how those motifs are represented in the brain.
5) To date no study has used multi-site local field potential recordings to characterize how a circuit's functional connectivity is modulated by social dominance (Aim 4).

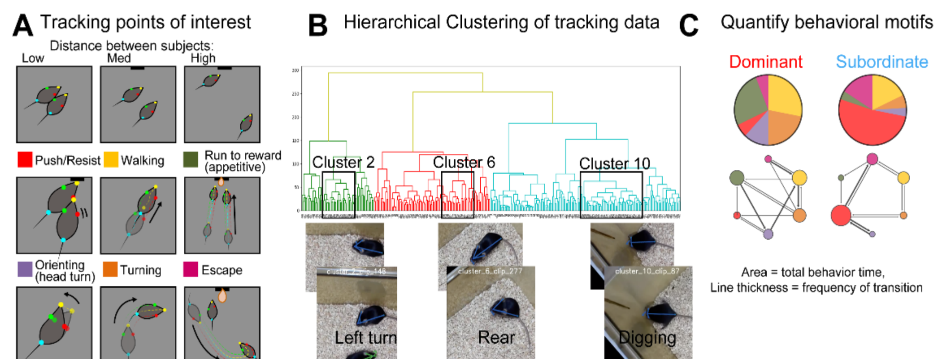

Figure 3: Pipeline to build behavioral profile for dominant and subordinate mice during reward competition. A. Using Deep Learning tools I will track point of interests in mice during the reward competition to identify behaviors of interest (e.g. indicated in squares). B. Hierarchical Clustering will reveal clusters of interest that will correspond to behavioral motifs. Shown, example hierarchical clustering from homecage data as a proof of principle successfully clusters behaviors based on pose tracking. Example clusters are left turn, rear and digging. C. I will quantify frequency of motifs and perform network analysis of the behavioral motifs for dominant and subordinate mice.

Approach

Aim 1: Characterize the behavior of dominant and subordinate mice in a reward competition task

Rationale/Preliminary Data: Our understanding of the neural basis of social behaviors is currently limited by the tools and assays used to characterize murine social behavior. In addition, existing social dominance assays for mice[16] lack the trial structure that benefits statistical analyses for neural activity. In order to identify how mPFC encodes social dominance I must have better quantitative measurements of behavior to link neural activity to those behavioral states. To overcome this challenge, I developed a trial based social competition assay where mice compete for a reward. My preliminary data show that dominant mice, as defined by the tube test, win most rewards across trials and occupy the reward port more often than subordinates (**Fig 2**). The goal of this aim is to use automatic tracking during this task to build a detailed profile of how behavioral motifs differ in dominant and subordinate mice.

Experiment 1: Track behavior in mice of different social ranks competing for rewards. All mice in this study will be group-housed with same age and sex and kept in a reverse light-cycle and groups will consist of 12 mice based on power analysis. Following existing protocols[16] I will use the tube test to rank mice. Once cages have stable social ranks, I will food restrict and individually train mice to associate a tone with a palatable reward consisting of 10 uL of Ensure. Mice will be trained for 8 days, or until all individuals within a cage are performing to the same criteria (100% of rewards picked up within to 10 seconds from cue onset; average latency to pick up reward <5 seconds). Once mice have reached learning criteria they will compete in the reward competition. The reward competition consists of dyadic competitions of 20 trials per session. Mice will be identified with bleach patterns in their fur and high-quality video will be taken during the competition. Deep Learning tracking methods DeepLabCut[33] and AlphaPose[34] will be used for tracking the location of both mice (**Fig 3A**). Since two mice could be at equal proximity to the reward port during reward delivery, a human observer will further inspect who consumed the reward across trials. Using unsupervised machine learning, specifically hierarchical clustering[35], I will cluster based on the pose tracking data (x,y position and body contour, length of body, length of head) to determine what behavioral motifs occur during the competition. Preliminary data suggests we can successfully track multiple mice and cluster their behavior in the homecage (**Fig 3B**). After clustering, I will use network analysis to determine how those behavioral motifs relate to each other in dominant vs subordinate mice, specifically quantifying transition probabilities and node connections between motifs (**Fig 3C**).

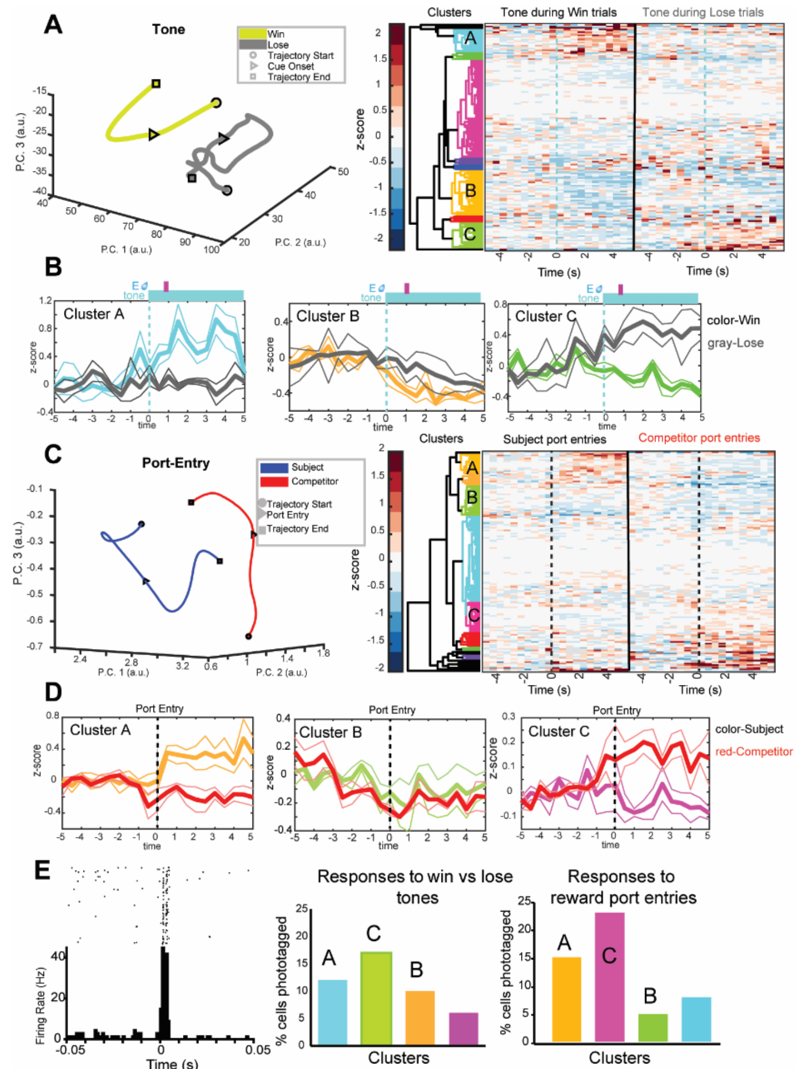

Figure 4: mPFC and mPFC-LH encodes task-relevant information during the reward competition assay. A. Left, neural ensemble dynamics of mPFC (neural trajectories formed by PCA using 5 sec of baseline and 5 sec of tone or port entry) during tones across winning and losing trials. Right, dendogram and zscores for individual mPFC cells (based on hierarchical clustering) during tone for winning and losing trials. **B.** Average zscore of single-unit firing rate during winning and losing for three example clusters identified with letters and colors. Gray lines are losing trials and color lines are winning trials. **C.** Same as A calculated for ensemble responses to port entries of subject vs competitor. **D** Same as B for single-unit responses to port entries of subject vs competitor. **E.** Left, example $mPFC^{LH}$ projector cells phototagged. Right, % phototagged cells out of total cells found in selected functional clusters for win vs lose and port entry responses (bar color and letter indicates identity in dendogram). Total recorded cells n= 152 phototagged cells n= 14. Data collected by N.P.C. Blue bar indicates tone. Dash line indivcates onset of tone or port entry. These data was recorded from wireless devices.

Predicted outcome 1.1*:* I expect that dominant and subordinate mice will have unique behavioral motifs, as identified by hierarchical clustering, which occur during the reward competition. I expect that some of the clusters only existing in dominant mice, and others exclusively in subordinate mice. Mice of intermediate ranks would show both types of behavioral motifs depending on who they are competing against. An alternative outcome is that no behavioral motif is unique to a given social rank, but the transition probabilities and connections shown with the network analysis differ in dominant vs subordinates.

Pitfalls/Alternatives 1.1*:* It is possible that tracking two mice simultaneously with DeepLabCut will be difficult, since when mice are near each other there could be identity errors. In anticipation of this challenge, we have been collaborating with Dr. Sejnowski's team to add a temporal filter algorithm to correct for identity of the subject tracked. Preliminary data suggests I can track multiple mice successfully (**Fig 3**). Another potential challenge is the clustering algorithm not segmenting behavior adequately (e.g. mixing multiple behaviors in the same cluster). In this case, we can use a combination of supervised labeling of behavior with the unsupervised clustering. Another approach would be to use manifold learning to identify distinct latent spaces and obtain their trajectories and characterize the behavioral motifs associated to those latent spaces. Importantly, I can also use conventional methods to analyze behavior via trained human observations, hence any technical challenges associated with the tracking methods will not impede the completion of this aim.

Aim 2 Test the hypothesis that the mPFC-LH circuit encodes social dominance via vGLUT and vGAT LH subpopulations.

***Rationale/Preliminary Data**:* The mPFC has been long implicated in social dominance behaviors across species, but there has been no characterization of how endogenous mPFC activity encodes social dominance. Furthermore, it remains unknown which mPFC outputs modulate dominance behavior. The LH receives a dense projection from the mPFC[36]and has vGAT and vGLUT subpopulations that control motivated behaviors in opposing manner[23,25]. Our preliminary data shows that mPFC and specifically the mPFC-LH circuit encodes social competition. The mPFC ensemble activity represents winning and losing (**Figure 4A-**B) and reward port entries by the subject vs the competitor (**Figure 4C-D**), and a high proportion of $mPFC^{LH}$ projectors represent the actions of the competitor (**Figure 4E**). Using the reward competition assay (Aim 1) I will characterize mPFC and $mPFC^{LH}$ activity across social ranks (Exp. 2.1-2) and I will characterize the anatomical and functional connectivity of mPFC with vGAT and vGLUT subpopulations in the LH (Exp. 2.3-4).

Experiment 2.1 Test the hypothesis that mPFC encodes competition-related information and social rank: Mice will be group housed with same sex and age matched cagemates, and implanted with a bundle of 32 single wires in the mPFC. After recovery, mice will be tube ranked and reward trained as indicated in Aim 1. Using wireless electrophysiology, I will record mPFC single units for 1 hour during the reward competition and will analyze the behavior as indicated in Aim 1. I will target recordings to intermediate ranked mice perform in three conditions: alone (no cagemate), against a dominant or subordinate cagemate. Importantly, I will have sessions in which half of the session (20 trials) will be against a dominant and the other half will be against a subordinate counterbalancing the order. I addition, I will have sessions in which half of the session will be performing the task alone and the other half in competition. This design will allow within session comparison across social ranks and compared to a non-social state (alone).To distinguish between the encoding of social rank vs mouse identity, I will repeat the assay under temporary chemical castration of the conspecific; this manipulation will maintain identity while disrupting dominance [37,38]. I will do analyses at the single cell level (firing rate comparisons; hierarchical clustering) and at the population/ensemble level (Principal Component Analysis; PCA) to identify what type of task-related information is encoded by the mPFC and how that changes when competing with dominant vs subordinates in intermediate-ranked mice. Using the approach of Aim 1 for analyzing the behavioral motifs I will compare mPFC ensemble and single-cell activity across all behavioral motifs to identify motifs that are most linked to mPFC activity.

Potential Outcome 2.1: Considering the preliminary data and previous literature showing that mPFC is more active in dominant mice, I expect that in dominant states there will be more cells task-relevant responses such as modulation by tones, port entries and other behavioral motifs associated with dominance (e.g. pushing, displacing from reward port). Another possibility is that there is the same amount of task-responsive cells in dominant vs subordinate states, but the firing patterns are distinct.

Experiment 2.2 Test the hypothesis that $mPFC^{LH}$ projectors encode competition-related information and social rank: I will selectively record $mPFC^{LH}$ projectors, by expressing Channelrhodopsin-2 (ChR2) in a cre-dependent manner by injecting the retrogradely traveling viral vector canine adenovirus carrying Cre-recombinase (CAV2-Cre) into the LH to allow for ChR2 expression (AAV5-DIO-ChR2-eYFP) in the subpopulation of mPFC neurons that project to LH. Four weeks after viral injection mice will be implanted with a 32-channel optrode. After recovery mice will be tube ranked and recorded in the reward competition as described in Exp. 2.1. $mPFC^{LH}$ projectors will be photoidentified (phototagged) based on short latencies and low jitter light responses[39].

Potential Outcome 2.2: I expect that $mPFC^{LH}$ projectors will be most active during winning trials as compared to losing or during alone trials (no cagemate), or that distinct subpopulations will represent winning, losing and alone conditions, suggesting they encode competition. I also expect that $mPFC^{LH}$ projectors will have a higher percentage of task-responsive cells compared with non-projectors.

Pitfalls/Alternatives for 2.1 and 2.2: If recording the same cells across different social rank states within the same session was prohibitive I can turn to single-cell calcium imaging in order to track the same cell across recording sessions. However, single-cell calcium imaging would not allow us to record both mPFC-LH projectors and non-LH projector mPFC cells within the same animal.

Experiment 2.3 Test the hypothesis that mPFC stimulation impacts vGAT and vGLUT LH subpopulations differently. In vGAT::Cre or vGLUT::Cre mice I will inject Cre-dependent GCamP6m (AAV5-CAG-flex-GCamP6m) and CAV-flp into the LH, and implant a GRIN lens in the LH for calcium imaging of these subpopulations using a miniature microscope. In addition, AAV9-fDIO-chrimson-mCherry or control virus will be injected in the mPFC to express chrimson or fluorophore in $mPFC^{LH}$ projectors. After recovery mice will be tube ranked and recorded in the reward competition as described in Exp. 2.1 using the nVoke miniature

microscope (Inscopix) I will image vGAT or vGLUT subpopulations while stimulating mPFC terminals. Our lab has successfully used the nVoke system to do optogenetics and calcium imaging simultaneously[40].

Potential Outcome 2.3: I expect that in dominant mice mPFC activation will evoke higher activity (more cells active or more calcium events) in vGAT subpopulations while in subordinate mice it will evoke higher activity of vGLUT subpopulations during the reward competition. Alternatively, in mice of intermediate rank, a distinct subpopulation of vGLUT and vGAT cells may be active during states of dominance vs subordination, supporting a more complex model than the one proposed in this grant.

Pitfalls/Alternatives 2.3: Calcium imaging may not capture baseline activity differences rather than tone-induced or behavior event-related. An alternative approach to this experiment would be phototagging vGLUT or vGAT cells using Chronos (modified opsin that allows for co-activating with Chrimson separate populations) while using Chrimson in mPFC somata.

Experiment 2.4 Characterize mPFC anatomical connectivity to LH vGAT and vGLUT: Recent advances in viral tracing techniques[41,42] allow determining if mPFC monosynaptically innervates vGAT and vGLUT LH subpopulations. Using Cre-lines for vGAT and vGLUT, I will inject modified rabies[42] into the LH of dominant or subordinate mice. After sufficient time for viral expression, mice will be sacrificed and mPFC tissue will be histologically processed. I will quantify the number of cells in mPFC projecting to vGAT and vGLUT populations. This experiment will provide a 2x2 design: dominant or subordinate, and vGAT or vGLUT. The outcomes will be number of rabies cells in mPFC per condition.

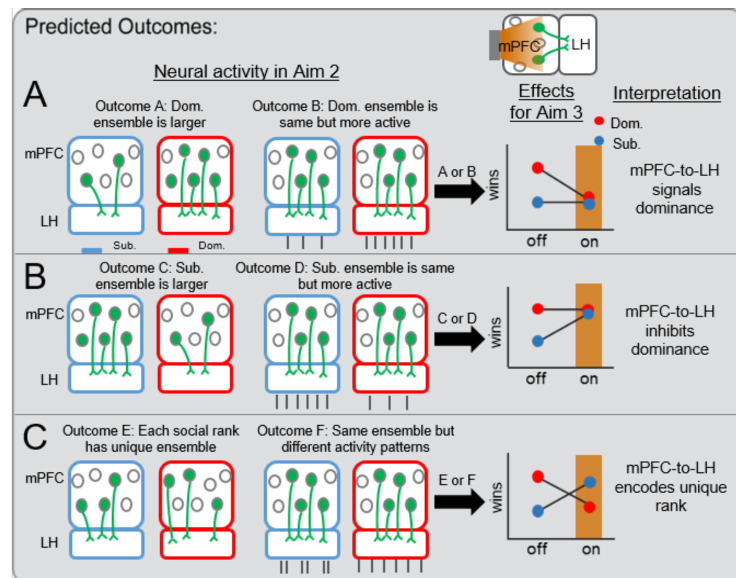

Figure 5: Predictions for Aim 3 based on the outcome of Aim 2. Interpretation and predicted behavioral effects of inhibiting mPFC-to-LH projectors based on the neural activity outcomes from Aim 2. **A.** activity of Dominant (Dom.) ensemble is more active or recruits more mPFC-to-LH cells. Thus, silencing decreases wins in Dom. animal. **B.** Subordinate (Sub.) ensemble is more active or recruits more mPFC-to-LH cells. Thus, silencing increases wins in Sub. animal. **C.** Sub. and Dom. ensembles have unique cells or activity patterns. Thus, silencing disrupts both social rank states.

Potential Outcome 2.4: I hypothesize that dominant mice will have more mPFC cells that project to vGAT LH cells than subordinate mice. This would provide a mechanism by which mPFC can drive approach and motivated behaviors stronger in dominant mice by recruiting more vGAT LH cells in dominant mice. Alternatively, there could be equivalent amount of mPFC cells innervating both subpopulations, which would suggest a more dynamic model where the same mPFC cells are recruited differently depending on rank.

Pitfalls/Alternatives 2.4: Our lab has successfully performed modified rabies tracing in the past. However, should issues arise, Dr. Callaway, who invented this rabies tracing approach, is well equipped to provide support. To account for differences in rabies viral expression across mice I will normalize to starter cell number in the LH[43]. Most LH neurons are either positive for vGAT or vGLUT so I expect that mPFC will project to at least one of these subpopulations. However, if it does not, I will shift Exp. 2.3 and 2.4 to the orexin LH subpopulation since there is already evidence that mPFC innervates these cells[44].

Aim 3: Test the hypothesis that mPFC-LH activity modulates social dominance behavior.

Rationale: In order to test if $mPFC^{LH}$ projector activity is sufficient or necessary for dominance behavior expression, I will use optogenetics to excite or inhibit this population during the reward competition. My preliminary data shows that optogenetic activation of $mPFC^{LH}$ projectors increases dominance behaviors during social competition.

Experiment 3.1 Inhibit $mPFC^{LH}$ projectors during the reward competition: To selectively inhibit $mPFC^{LH}$ projectors I will use the dual virus approach to express the light-activated chloride pump halorhodopsin (eNpHR3.0)[45] in mPFC cells projecting to LH. To this end, I will bilaterally inject AAV-DIO-eNpHR3.0-eYFP into the mPFC coupled with CAV2-Cre into the LH. In addition, I will bilaterally implant fiber optics into the mPFC to deliver light locally. A control group of mice will be treated in the same way except that they will be injected with AAV-DIO-eYFP into the mPFC. Two months after viral injections, mice will be trained in the reward competition assay, and tested with alternating light off and on every 5 min in the reward competition assay. Mice will be tested against a dominant and subordinate conspecific from the same cage, such that $mPFC^{LH}$ inhibition is performed with both a higher and lower-ranked competitor. To have a secondary dominance assay, I will also perform the tube test to quantify how much behavior is affected by $mPFC^{LH}$ inhibition. Since the mPFC is

involved in effort I will use an effort-based Tmaze to control for any changes in effort that mPFC^{LH} manipulation might have. To control for locomotion, general effort, and feeding effects, I will inhibit mPFC^{LH} projectors during the open field, effort-based T-maze (mice choose between high effort/high reward vs low effort/low reward choice) and in a homecage feeding assay. Mice will be identified with bleach patterns in the fur and high quality video will be taken for post-hoc processing using the automated tracking and behavioral motif classification as in Aim1.

Potential Outcomes 3.1: I will be able to make predictions for this experiment based on the data I observe in Aim 2. First, if the ensemble that encodes dominance is larger in size or more active, then I expect that mPFC^{LH} inhibition will decrease dominance behavior expression (**Fig 5A**). Alternatively, if the ensemble that encodes dominance is smaller in size or less active, then I expect that mPFC^{LH} inhibition will increase dominance behavior expression (**Fig 5B**). Interpretations and other outcomes are detailed in figure 5.

Experiment 3.2 Activate mPFC^{LH} projectors during the reward competition: To selectively excite mPFC^{LH} projectors I will use the the same viral approach described in Exp. 3.1 to express AAV5-DIO-ChR2-eYFP. Two months after viral injections, mice will be trained in the reward competition assay, and tested with alternating light off and on every 5 min pulsing the light at 5 Hz in a bursting manner during the reward competition assay. Behavioral data processing and control experiments will be done as described in Exp. 3.1.

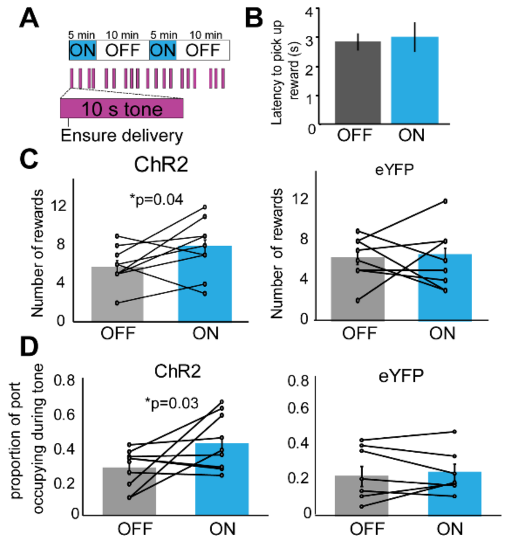

Figure 6: mPFC-LH stimulation increases social dominance. A. Light stimulation parameters used during the reward competition. B. Stimulation while performing reward task alone did not change latency to pick up. ChR2 n=8 C. Number of rewards obtained increased during mPFC-LH stimulation (ChR2 n=10, eYFP n=8). D. Proportion of reward port occupation increased with stimulation. Data collected by N.P.C.

Preliminary Data 3.2: Optogenetic activation of mPFC^{LH} projectors in subordinate mice during the reward competition increased number of rewards obtained, but did not affect the latency to enter the reward port while performing the task alone (**Fig 6**). Importantly, photoactivation of mPFC^{LH} projectors did not change distance traveled in the open field, social preference for a novel mouse, feeding behavior nor did it changed effort, mice did not change the percent of high reward/high effort choice (data not shown).

Expected Outcomes 3.2: Considering the preliminary data, I expect that activation of mPFC^{LH} projectors will not only increase winning, but also increase the frequency of behavioral motifs associated with dominance.

Pitfalls and Alternatives 3.1 and 3.2: It is possible that mPFC^{LH} projector activity is necessary for generating some motor behaviors, rather than expressing dominance. To address this caveat, I will examine behavioral effects of optogenetic manipulations in the reward task while animals perform the task alone and in the open field to distinguish social behavior effects from generalized motor or locomotion effects. Moreover, it is possible that short latency inhibition achieved with optogenetics is insufficient to change the dominance expression. In this case, I would turn to design receptors exclusively activated by designer drugs (DREADD) to inhibit mPFC^{LH} for hours instead of minutes. Finally, if there are no effects of mPFC^{LH} modulation on dominance behavior, I will explore other mPFC outputs such as the nucleus accumbens and basolateral amygdala considering their roles in reward and valence respectively they could potentially modulate dominance. Importantly, I have evidence that not all mPFC outputs affect social dominance since mPFC projections to the dorsal raphe nucleus does not change dominance behavior during the reward competition (data not shown).

(R00) Aim 4: Testing the hypothesis that social memory is upstream of social dominance encoding.

Rationale: Social dominance expression could rely on memories of previous social interactions, or alternatively, dominance could be social memory independent with social cues indicating rank. Considering that the direct connection between vHPC to mPFC is necessary for social memory[28], I will test the hypothesis that vHPC input to the mPFC is required for social dominance encoding and expression. Furthermore, the vHPC synchronizes with the mPFC at different frequencies depending on the behavioral state, thus providing a possible way to potentially encode dominance. The proposed experiments will test if, in the mPFC, encoding of social dominance exists in parallel with social memory circuits, or in series in a dependent manner. Moreover, they will test how vHPC input contributes to the functional connectivity between the mPFC and LH during social dominance.

Experiment 4.1 Determine if vHPC input is necessary for mPFC encoding of social dominance: I will unilaterally inject AAV-eNpHR-eYFP into the vHPC and implant a single-unit electrode ipsilaterally into the mPFC. Since vHPC input to mPFC is ipsilateral, this design will allow to disrupt vHPC input while preserving behavior. I have successfully done this approach for this pathway during other behaviors[3]. After recovery from surgery, mice will be trained and recorded during the reward competition as indicated in Experiment 2.1.

During recording sessions yellow light will be delivered during randomized trials. To account for non-social task-related information I will also inhibit vHPC input while recording during reward alone sessions.

Expected Outcomes 4.1: I expect that inhibiting vHPC will increase the similarity of mPFC activity during dominant and subordinate states at the level of the ensemble and single-unit activity or decrease task-relevant activity (e.g. win vs lose differences), thus abolishing mPFC's representation of social dominance. This finding would be consistent with vHPC input providing necessary information, likely social identity, for the mPFC to represent social rank during social competition.

Experiment 4.2 Determine if vHPC input is necessary for expression of social dominance: I will bilaterally inject AAV5-eNpHR3.0-eYFP into the vHPC and implant bilateral fibers into the mPFC. I will inhibit vHPC-mPFC during the reward competition following the experimental protocol in Exp. 3.1 alternating light off and on periods.

Expected Outcomes 4.2: I hypothesize that bilateral inhibition of vHPC input will change the outcome of the reward competition, specifically disrupting the priority access to rewards that dominant individuals have, and decreasing differences seen between dominants and subordinates in the frequency of behavioral motifs. Alternatively, it is possible that inhibition of vHPC does not affect the outcome of the competition, which would be consistent with social memory not being necessary for dominance expression.

Pitfalls and Alternatives Experiments 4.1 and 4.2: It is possible that short latency inhibition achieved with optogenetics is insufficient to disrupt social behavior/encoding in the reward competition. In this case, I would turn to DREADDs expressed in vHPC cells that project to mPFC using the CAV2-Cre approach, which would allow to inhibit for hours instead of minutes. Previous work has shown that inhibition of vHPC-mPFC results in decreased anxiety-like behavior[3], since I are inhibiting both mice for Exp. 4.2, I won't bias behavior by reducing the anxiety-like behavior in only one mouse. The vHPC-mPFC circuit is not the only pathway involved in social memory, thus it is possible that mPFC uses an alternative circuit to retrieve social memory information for appropriate social rank expression. A potential alternative circuit to probe is the projection from vHPC to the Nucleus Accumbens which is also necessary for social memory[26].

Experiment 4.3 Determine if vHPC input modulates social dominance mPFC-LH functional connectivity. The vHPC provides direct input to both mPFC and LH but it is unclear if these anatomical connections carry functional information during dominance expression. To understand how vHPC activity modulates the functional connectivity of the vHPC-mPFC-LH circuit during social dominance, I will use multi-site local field potential (LFP) recordings combined with optogenetic inhibition of vHPC activity. I will implant electrodes in the vHPC and LH, inject AAV5-eNpHR3.0-eYFP into the vHPC and implant a fiber optic in the mPFC. After 8 weeks of viral expression, I will record LFP activity during the reward competition task in dominant and subordinate mice. I will quantify and compare vHPC-mPFC-LH synchrony (coherence, cross-frequency coupling) and power with and without vHPC-mPFC inhibition in dominant vs subordinate mice to identify functional signatures related to dominance and how they are affected by vHPC inhibition.

Expected Outcomes 4.3: I expect that dominant mice will have stronger mPFC-LH synchrony during the reward competition compared with subordinate mice and that mPFC-LH synchrony will decrease with vHPC-mPFC inhibition. Alternatively, mPFC-LH synchrony could be the same across social ranks, but be disrupted at distinct frequencies for dominant vs subordinate mice. Finally, it is possible that any mPFC-LH synchrony disruptions are the same across social ranks, suggesting that vHPC input is equally important in dominant and subordinate states.

Pitfalls and Alternatives 4.3: The vHPC projects directly to the LH, if I do not see any changes in synchrony, I could repeat the experiment but silence vHPC somata to disrupt vHPC input to both mPFC and LH. In addition, although LFPs are often recorded from mPFC and vHPC, regions with strong oscillations due to the layer structure, I recognize that it is uncommon to record LFP in subcortical regions and if there was any problem with attempting to record LFP from LH I will record single units from LH while simultaneously recording LFP from mPFC and vHPC and will use LFP-spike metrics to quantify synchrony.

Note: For all experiments in this study, if no sex differences are detected in this study, the data for females and males will be pooled (see Vertebrate Animals). Considering that dominance behaviors differ in female and male mice[46], it is possible that there will be some sex differences in this study. In this case, I will dissect the mechanisms of those sex differences as part of my independent work, by continuing my collaboration with Dr. Curley who has the appropriate expertise.

Future Directions: Altogether, this work will inform how neural circuits may be disrupted during social deficits in psychiatric patients. Moreover, Aim 4 (R00 phase) will generate follow up experiments for my R01 such as using single-cell calcium imaging to study vHPC encoding during dominance and rabies tracing studies to determine if mPFC cells that project to LH receive vHPC input. Furthermore, I can use the technical approaches I learn in my K99, as well as my expertise in multi-site electrophysiology, to probe the role of different mPFC outputs in social dominance.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Responsible conduct of research is a top priority both for myself and my postdoctoral mentor, Dr. Kay Tye. Our research group puts a special emphasis on facilitating training, discussion, and professional development to maintain high standards for safety and ethics in our research. Throughout the K99 and R00 phases of this award, I will participate in a number of courses and meetings to ensure these standards are upheld for the proposed research program and in my future career, as well as with my future trainees.

1.'Responsible Conduct of Research/Ethics and Survival Skills in Academia' course at UCSD
Format: Formal course (lectures, coursework, face-to face discussion)
Frequency and Duration of Instruction: Weekly 1 hour lectures, 10 weeks total
Faculty Participation: Yes, instructor Dr. Michael Kalichman, Director of the Research Ethics Program, as well as faculty guests and panel members for discussion of research ethics.
Subject Matter: This course is provided by the nearby University of California San Diego (UCSD), and available to all members of the Salk Institute for Biological Studies. The content of this course is specifically designed to meet current NIH and NSF requirements for training in responsible research conduct. Course subjects include training on reproducibility of data, peer review ethics, recognizing research misconduct, whistleblowing, and sexual harassment. The course format consists of a core of 10 weekly 1-hour lectures, which are supplemented by web-based reading and assignments, written assignments, and participation in roundtable and 1-on-1 discussion groups. I will complete training in this course once during the K99 period of the award and conduct training in a course of similar breadth and duration no fewer than once every three years during the R00 phase of the award and throughout my future career.

2. Group meetings on ethics in research and academia
Format: Discussion groups (face-to-face discussion with presentations from participants)
Frequency and Duration of Instruction: Twice a year 2 hour group meetings (entire K99 phase)
Faculty Participation: Yes, Dr. Kay Tye, and Visiting Scholar faculty members from other institutions
Subject Matter: Our lab also organizes weekly 2 hour specialized discussions ('subgroup meetings') to discuss technical, conceptual or ethical subjects. Each year several of these meetings are devoted to responsible conduct in research topics including: standards/transparency in analyzing, data management, reporting and sharing, professional conduct in a research setting (interpersonal conflicts and sexual harassment), ownership of scientific research, mentoring, peer-reviewing, authorship, ethics in animal research (surgical procedures and behavioral experiments) and many others.

3. Ethics discussions during yearly science retreats
Format: Discussion groups (face-to-face discussion of selected ethic topics)
Frequency and Duration of Instruction: 6 hours Once a year (entire K99 phase)
Faculty Participation: Yes, Dr. Kay Tye, and Visiting Scholar faculty members from other institutions
Subject Matter: Our lab also organizes yearly retreats where we leave lab and for a few days discuss different important topics related to science. One of the major topics discussed is ethics such as mentoring, whistleblowing, ethics related to animal research, diversity in science and social responsibilities of scientists.

4. Training in responsible conduct of animal research (several different classes/sessions)
Format: Online courses, wet labs, and instructional seminars
Frequency and Duration of Instruction: Courses and training will be taken and refreshed annually, with additional training as needed for any new animal procedures (surgical or behavioral) used in research.
Faculty Participation: Senior staff scientists and veterinary staff at the Salk Institute
Subject Matter: Ethical conduct and adherence to legal requirements for animal research is an absolute requirement for my research. The Animal Resources Department at the Salk Institute will provide training in proper sterile technique, postsurgical monitoring and care, registration and monitoring of food restriction in compliance with IACUC standards.

Statements by Mentors and Collaborators

Mentor: Kay M. Tye, Ph.D.

1. Mentor-Trainee Match and Training Goals

I am thrilled to provide my absolute strongest support and commitment as the primary Mentor for Dr. Padilla-Coreano's application. From the moment I met Dr. Padilla-Coreano, she immediately stood out to me as bold, deep, and innovative. I worked for almost 2 years to recruit her to my laboratory and I'm delighted that she joined our lab 3 years ago. I continue to be impressed with the remarkable talent that Dr. Padilla-Coreano shows for original and creative thought while maintaining a high level of skepticism and rigor. Since joining my laboratory she has developed new paradigms, received numerous fellowships, established new technology in the laboratory and co-authored two articles, including a first-author paper in Neuron in 2019. During this past year she has collected very exciting datasets that I know will lead to a solid and impactful publication. She is attracted to the biggest, most challenging and important questions of our field and is both fearless in tackling them and extraordinarily capable of doing so in a rigorous, creative and quantitative manner. She is a great leader and a visionary. She is a force of nature: she would bloom on an iceberg, and quite frankly, STEM needs many more people like her in it. While anyone would consider her highly productive, what she has accomplished is even more impressive when considering that she tackled an extremely ambitious "blue sky" project in my lab, and is consistently active in outreach. When considering the delta from starting points, current positions and future trajectories, I would rank her one in ten thousand (top 0.01%). Without considering those factors, she still lands in the top 1%. The research and training plan in this application will launch Dr. Padilla-Coreano on a meteoric trajectory for a wildly successful career as an independent investigator, a field leader, and a champion for the underrepresented communities.

<u>Rationale for additional training at the Salk Institute for Biological Studies</u>

Dr. Padilla-Coreano is an ideal candidate for the K99/R00 funding mechanism. She has a strong track record of productivity, and has developed an excellent training plan and timeline to transition to independence. Her goals are ambitious, but achievable and I am completely dedicated to actively support, advocate and facilitate all aspects of her training and career development. The training plan we have crafted together includes a comprehensive plan to achieve Dr. Padilla-Coreano's goals to develop new expertise, improve existing expertise, and develop skills necessary for independently leading a group. Dr. Padilla-Coreano's training includes **1)** Activities to foster her intellectual development so that she can crystallize a unique conceptual framework to launch her own research program **2)** Training to equip her with the necessary skills and expertise to execute her research program, and **3)** Experiences to prepare her to effectively and ethically lead and mentor her own research team and pass her knowledge and expertise down to the next generation of neuroscientists.

During two additional years in the lab she will work on the following specific training and career goals:

a. <u>Develop expertise in computational approaches for behavioral analysis</u>: During the past 15 years I experienced the transformation of systems neuroscience by the use of optogenetics. Particularly, using optogenetics to manipulate specific projections, pioneered by myself and my postdoctoral advisor Karl Deisseroth, has allowed us dissect neural circuits. Dr. Padilla-Coreano and I believe that the next transformation of our field will be due to machine learning transforming behavioral neuroscience. For Dr. Padilla-Coreano's K99 mentored phase she will develop the skills to effectively apply existing machine learning tools for behavioral analysis. Towards this goal Dr. Padilla-Coreano will benefit from the guidance of Drs. Terry Sejnowski and James Curley and will take two courses at UCSD (COGS 118A and COGS 18) that will provide formal training in python and machine learning.

b. <u>Expand repertoire of *in vivo* techniques to interrogate neural circuits</u>: Dr. Padilla-Coreano is already an expert on *in vivo* electrophysiology, she will use her training time to expand her knowledge on circuits neuroscience by learning in vivo epifluorescent calcium imaging and viral-tracing for circuit mapping. Towards this goal Dr. Padilla-Coreano will benefit from the additional guidance of Dr. Ed Callaway.

c. <u>Improve leadership and management skills</u>: Although Dr. Padilla-Coreano has already had the opportunity to lead a small group (4-5 people) within my lab, I was heavily involved in advising her through specific challenges and exposing her to leadership and management theory/literature. I would now like to watch her apply what she has learned both from a scholarly review of leadership/management literature and empirical experience with less direct involvement from me (but under my watchful eye). By leading a team and receiving coaching from me in our one-on-one

meetings Dr. Padilla-Coreano will transition from proficiency to mastery in mentoring and leadership. Additionally, Dr. Padilla-Coreano will attend workshops on management and leadership offered by the Salk Institute.

d. Scholarly development: By attending and presenting her research at national and international conferences, as well as attending and presenting in the laboratories of her advisors and collaborators, Dr. Padilla-Coreano will develop her scholarship and communication skills.

Kay M. Tye's Research Background and Mentor Experience:

Training the next generation of research scientists is my greatest passion, and though I am relatively early in my career I believe I already have an outstanding record of mentoring excellence. Before the move to our current location at the Salk Institute, I first established my independent research lab in Jan 2012 at the Massachusetts Institute of Technology (MIT) and received tenure there in 2018. In less than 8 years of having my own lab, five postdoctoral trainees under my supervision have succeeded in obtaining tenure-track positions in top research institutions. Dr. Anna Beyeler, had a stellar funding and publication track record (Nature, Neuron, Cell Reports) and established her lab in 2017 at Neurocentre Magendie in Bordeaux, France. There she has already been highly successful in securing competitive operating grants for her research program. Dr. Cody Siciliano, has successfully transitioned to a tenure-track faculty position at Vanderbilt University in August 2019. While in my lab, Dr. Cody Siciliano received a K99/R00 research award, and had excellent publications (Nature and Science). Similarly, Dr. Anthony Burgos-Robles also started a tenure-track faculty position at the University of Texas in San Antonio. Dr. Gwendolyn Calhoon is now faculty at Bates, and Dr. Stephen (AZA) Allsop is now in a faculty-equivalent physician/scientist position at Yale. In the 6.5 years I was at MIT, I graduated 5 students -- all of whom have published well and have all succeeded in winning funding from external agencies including the NIH, NSF, and other competitive foundation grants. Furthermore, for my record of mentoring and teaching at MIT, I was recognized with the MIT Graduate Mentoring Award, the BCS Award for Excellence in Undergraduate Advising, the Outstanding Undergraduate Research Faculty Mentor Award, the MIT 'Commitment to Caring' Award, and the 2018 Award for Outstanding Postdoctoral Mentoring. **Consequently, I have experience successfully mentoring trainees through their transition to independence as well as under the K99/R00 funding mechanism, and will be able to provide expert guidance to Dr. Padilla-Coreano as she proceeds to navigate the transition to independence.** This record of mentorship, combined with the hands-on training and availability I will be able to provide for Dr. Padilla-Coreano, will ensure that she has maximum possible support to achieve the training and career goals outlined in this application.

In addition, we have also assembled an outstanding mentorship committee at the Salk Institute to further help Dr. Padilla-Coreano reach her training plan. This will consist of Dr. Terry Sejnowski and Dr. Ed Callaway (see respective Letters of Collaboration), both faculty members at the Salk Institute. They will work closely with Dr. Padilla-Coreano to provide her with specialized training and career guidance as she completes her mentored phase work and transitions to an independent research position.

Advisory Committee and Collaborators Team:

We have assembled a team of world-renowned advisors and experts to ensure that Dr. Padilla-Coreano not only meets her goals but excels at them. Dr. Padilla-Coreano will receive research training, technical support, feedback on publication, and career advice through meetings with her Advisors and Collaborator. Her Advisors Drs. Terry Sejnowski and Edward Callaway have committed to support Dr. Padilla-Coreano's career.

Dr. Padilla-Coreano will be presenting in Callaway and Sejnowski lab meetings and having one-on-one meetings with her advisors, which will allow her to improve her communication skills and get valuable scientific feedback and career guidance. My study is immediately adjacent to Terry Sejnowski's and my lab is immediately next to Ed Callaway's lab, and their close proximity allows for lots of interaction! The committee and myself represent a diverse group of research expertise at varying academic stages, which will provide Dr. Padilla-Coreano with interdisciplinary advice and perspectives. Dr. Callaway, is a pioneer on circuit-mapping, tool development (particularly monosynaptic rabies tracing), and has characterized the visual system in mouse models. Dr. Sejnowski is a world-renowned expert on Neural Networks and Computational Neuroscience, and his seminal work on Neural Networks and Deep Learning changed the field. Importantly, Dr. Padilla-Coreano's advisory team has an excellent track-record transitioning postdocs to independence.

In addition, Dr. James Curley will serve as a collaborator and mentor. Dr. Curley is a world-renowned expert in social dominance in mice and in statistical methods to analyze behavior, including social network analyses.

Dr. Padilla-Coreano and I have had a professional and intellectual relationship with Dr. Curley for the past 3 years. We have had multiple conversations that shaped beginning stages of the work proposed on this K99 application. Dr. Padilla-Coreano has visited his Lab and we have been in communication about our research progress in all projects related to social dominance.

Together with this excellent team, I will execute the career development plan detailed below.

2. Career Development Plan:

Consistent with NIH goals, Dr. Padilla-Coreano will receive an interdisciplinary, multilevel training that will range from computational, behavioral to neural circuit approaches and professional development training.

Develop expertise in applying computational approaches for behavioral analysis: Dr. Padilla-Coreano has strong expertise in behavioral neuroscience, but one gap is her lack of knowledge of computational tools that facilitate and enrich behavioral analysis. The top goal for Dr. Padilla-Coreano's K99 phase of this award will be to develop expertise in the use of machine learning approaches to automatically track and classify behavior, and to do social network analysis of behavior. Dr. Padilla-Coreano has started using DeepLabCut, a recently developed deep learning neural network, to track body position during social behaviors. Dr. Padilla-Coreano currently has background in programming, and some familiarity with Python, a common language used for deep learning and machine learning packages and tools. We have identified two key courses at UCSD (COGS 118A and COGS 18) for Dr. Padilla-Coreano to audit during her first year of the K99 training, which will provide formal instruction in Python and theoretical underpinnings of machine learning approaches. Under the guidance of Dr. Terry Sejnowski, she will use python libraries for unsupervised clustering of the tracked body positions to cluster the behavioral data into distinct behavioral motifs. We have proof of principle data that show that this approach is possible (Aim 1). Next, with the collaboration of Dr. James Curley, Nancy will use social network and other relevant statistical analyses that will allow her to identify social dominance differences, even if there are subtle changes in behavior, and how manipulating neural circuits changes behavior. Altogether learning these approaches will position Dr. Padilla-Coreano to become a successful independent investigator with the right tools to dissect the neural basis of social behaviors.

Expand repertoire of in vivo techniques to interrogate neural circuits: Dr. Padilla-Coreano developed a strong expertise in multi-site electrophysiology during graduate school. She is an expert in the use of local field potentials and single units to measure functional connectivity during behavior. This expertise will serve her well during her independence phase. However, considering the importance of studying subpopulations of cells to dissect neural circuits, she will be learning freely moving single cell calcium imaging and rabies tracing. Our lab has published several papers with single cell calcium imaging and Dr. Callaway will provide additional support for rabies tracing tools.

Intellectual and scholar development through meetings and presentations: A critical part of Dr. Padilla-Coreano's training will be ensuring she gets high-quality feedback on her research from members of her supervisory committee, her peers, and the greater scientific community. We have prepared a robust program of meetings and conferences detailed below that will ensure Dr. Padilla-Coreano has every opportunity to develop professionally and increase her visibility within the neuroscience field. I have also significantly reduced my travel for the foreseeable future to increase my time spent mentoring in the next few years. I detail some of the meeting and presentation opportunities Dr. Padilla will have below.

One-on-one meetings with Mentor- First and foremost, I will be fully available to Dr. Padilla-Coreano to give all possible support and direction during her research. Currently, we have one-on-one meetings on a biweekly basis, with additional meetings added as needed to deal with particular technical or conceptual issues that arise. During these meeting we discuss progress, data, setbacks and revise short- and long-term timelines based on any new developments in the project. As Dr. Padilla-Coreano progresses, the direction of these meetings will also shift to include planning for upcoming conferences, manuscript preparation and strategy, preparation for job applications, and interview preparation for tenure-track positions.

Meetings within the Tye Lab- A critical source of feedback for Dr. Padilla-Coreano during her research will be the regular lab meetings held in my research group. At our weekly lab meetings, Dr. Padilla-Coreano will frequently present her work to discuss her research progress, troubleshoot any technical issues that arise, and refine the presentation of her project's story. We also hold weekly subgroups for focused discussion on specific topics, ranging from theory to new tool development. An important regular subgroup topic is ethics in research, and we will discuss best practices concerning data preparation, animal welfare, scholarly conduct, and many other areas. Integrity and ethical conduct are top priorities in my research lab, and these meetings will help Dr.

Padilla-Coreano ensure that all her research is done to the highest professional standards in the field.

Attending Lab meetings of Supervisory Committee- Dr. Padilla-Coreano will attend lab meetings of Drs. Terry Sejnowski and Ed Callaway every other month. This will allow her to expand her expertise on computational approaches in Neuroscience (by participating in Sejnowski Lab meetings) and Circuit Dissection (by participating in Callaway Lab meetings). Importantly, both of these laboratories are in the same building as the Tye lab, facilitating constant communication between Dr. Padilla-Coreano and Sejnowski and Callaway Lab members. Dr. Sejnowski's group holds computational tea times every day at 3:30pm with intellectual and technical informal discussions about computational approaches to study the brain. Dr. Padilla-Coreano will attend tea times weekly to discuss ongoing progress with machine learning experts from the Sejnowski group. At the end of each mentored period year, Dr. Padilla-Coreano will present her research in the Sejnowski and Callaway Lab meetings to receive and incorporate feedback.

One-on-one meetings with Advisory Committee and Collaborators- Dr. Padilla-Coreano's advisory committee will maintain regular meetings with her advisors and collaborator to discuss research challenges and milestones, and provide support during her applications to tenure-track positions. Dr Padilla-Coreano will meet with Dr. Sejnowski as needed and with Dr. Callaway in person and with Dr. Curley via skype once a month during the initial stages of her training, with adjusting meeting frequencies according to her training needs.

Presenting on Scientific Meetings: During the K99 phase, Dr. Padilla-Coreano will present her research at 3 internationally-attended conferences per year. Specifically, she will attend 1) Society for Social Neuroscience's annual meeting, to get exposure and feedback from her immediate field (potential peer-reviewers and experts in the field), 2) Computational Systems Neuroscience (COSYNE) meeting to get exposed to quantitative methods used for behavioral neuroscience research and receive technical feedback on the machine learning methods she will be applying to her research, 3) the American College of Psychopharmacology (ACNP) which will expose her to translational perspectives to her research, and a networking opportunity with the extended community of behavioral neuroscientists. These meetings allow Dr. Padilla-Coreano to receive valuable feedback on her work from experts in the field, practice her communication skills, and develop her reputation in the neuroscience community. Moreover, as she gets ready to apply for jobs these meetings will allow her to network. In addition to these conferences, Dr. Padilla-Coreano will attend many different meetings at the Salk Institute and neighboring University of California San Diego (UCSD). These include the Salk seminar series (weekly), the 'First Friday' symposium series (monthly), the Neuroscience Seminar Series held at the UCSD Center for Neural Circuits and Behavior (CNCB) and the Institute for Neural Computation (INC) seminar series, among many others. Many of these local conferences will also provide opportunities for Dr. Padilla-Coreano to present her research findings to the San Diego neuroscience community.

Publishing: Publication of Dr. Padilla-Coreano's work will be the highest possible priority during the mentored phase. During her K99 training, Dr. Padilla-Coreano will prepare and submit a manuscript based on Aims 1 of her research proposal within the first year of the award, and a second manuscript based on Aim 2-3 early in the second year of her K99 award. This will allow time for any revision experiments necessary as she secures a position on the academic job market, allowing her to complete her mentored phase training and achieve independence within the timeframe of award support. Based on preliminary data Dr. Padilla-Coreano has collected and the potential importance of her findings, we anticipate this study will be an extremely high impact publication. Dr. Padilla-Coreano will also receive extensive support from myself and her advisory committee during the preparation of the manuscript. In addition to this study, I anticipate that Dr. Padilla-Coreano will co-author several publications considering the foundational work on social behavior she has done in my lab, and the training she is providing to others. Together with Dr. Padilla-Coreano's already impressive publication record, these additional first-author and co-author studies will make her a highly marketable candidate for tenure-track positions at top-tier research institutions.

3. Sources of Support:

Dr. Padilla-Coreano will have ample resources to support her research and training. My lab has been supported by multiple NIH grants and we have all of the equipment necessary for all of the proposed experiments in this application. The following funds will provide support for Dr. Padilla and all support personnel, animal costs, equipment and reagents needed during the mentored phase of her proposed research.

4. Mentor commitment to Dr. Padilla-Coreano (Nature/Extend of Supervision)

I am fully committed to ensuring the success of Dr. Padilla-Coreano during the K99/R00 award and her future career. Dr. Padilla-Coreano will have access to the full resources of my laboratory and the Salk Institute for her work, and I will be available to provide mentorship and guidance for all aspects of her research and career development. During our recent relocation to the Salk Institute, Dr. Padilla-Coreano has been a pillar of leadership in our research group. She not only helped with the coordination to transfer equipment to our new institute, she also took a leadership role in helping to interview new candidates applying to the lab to ensure we maintained a thriving research program as we transitioned to our new location, all while rapidly moving her own research project forward. The planning and leadership ability that Dr. Padilla-Coreano has shown during this time have been remarkable, and the move (and subsequent setup of our lab) have provided invaluable experience for setting up her own lab. Dr. Padilla-Coreano has been a huge asset to my research program, and I am fully committed to investing in her continued success. In addition to support for her research, Dr. Padilla-Coreano will be free to travel to any conferences, job interviews or other professional development opportunities needed during her time in my lab. I will also provide annual evaluations of Dr. Padilla-Coreano' progress during the mentored phase as outlined in FOA guidelines.

5. Responsibilities and Allocation of Time

I can confirm that Dr. Padilla-Coreano will be supported to devote 100% of her time to the proposed research and training program during the K99 training period. Dr. Padilla-Coreano will have no other formal duties such as teaching, membership on committees or administrative duties, unless these are voluntarily chosen for career development reasons and will not hinder her research. To maximize Dr. Padilla-Coreano' research time she will have extensive support from core facilities at the Salk Institute, in particular the expert staff at our animal facility who will handle all ordering, husbandry, and breeding of experimental mice used in her research at her direction. Procuring equipment and reagents will also be handled by senior administrative staff within the lab. During the mentored phase Dr. Padilla-Coreano will be responsible for the training and supervision of several members of my lab, but this will occur while they work together with her on her project and will therefore be a net benefit to her research productivity as well as an excellent opportunity for leadership skill development. Dr. Padilla-Coreano will also have anytime needed to participate in local, national and international meetings to present her work.

6. Transition to Independence and R00 Phase

One of my most important responsibilities during Dr. Padilla-Coreano's training will be providing guidance as she transitions to her independent career. As Dr. Padilla-Coreano prepares to enter the job market, she will receive personalized training from me and the other members of her supervisor committee in strategically finding and applying for jobs, preparing job application materials, and practicing her presentation skills for job interviews. We will also prepare a series of practice 'Chalk Talks' for Dr. Padilla-Coreano to hone her communication ability, and clearly convey the strengths of her research experience and future direction. Additionally, as outlined above I will ensure that Dr. Padilla-Coreano has access to resources and personnel support to successfully publish her first-author work during this time. **Dr. Padilla-Coreano's project studying the circuitry of social dominance was developed with her full intellectual leadership, and she will be free to pursue all future directions and offshoot projects from this work in her own lab. Importantly, her use of multi-site electrophysiology to use local field potentials (LFPs) to record long-range circuits during behavior is not an approach that I do in my laboratory providing her a unique and distinct niche to develop. Dr. Padilla-Coreano has expertise quantifying functional connectivity during behavior with LFPs providing her an opportunity to combine her graduate work experience with her postdoctoral training. In addition, Dr. Padilla-Coreano has a strong interest in studying how social dominance differs in females and males, a research avenue that is different from my lab's program. As Dr. Padilla-Coreano enters the R00 phase, I will continue to be available for guidance as she establishes her research program and begins to apply for competitive research funding and her first R01 grant. I will also provide advice on many other aspects of her new position, such as hiring personnel, purchasing equipment, navigating academia as a new faculty member and maintaining a culture of scientific integrity in her new lab.**

Dr. Padilla-Coreano has been an outstanding member of my lab, and she will have my full support during all phases of her training. The K99 phase research and training plan will equip her to direct a cutting-edge research program and be a highly sought after job candidate, and the proposed R00 phase experiments will allow her to establish an exciting and innovative direction for her independent career. I am fully committed to ensure that Dr. Padilla-Coreano has all possible resources and support needed to achieve her career goals.

Sincerely,

Kay M. Tye, Ph.D.

Crick-Jacobs Center for Theoretical and Computational Biology

September 17, 2019
K99/R00 Reference Letter

To Whom It May Concern:

I am writing to enthusiastically support to Dr. Nancy Padilla-Coreano in her K99/R00 award application as an advisory committee member during the mentored phase of this award. My primary role during this training period will be to ensure that Dr. Padilla-Coreano receives guidance and training needed to understand and leverage the advanced computational techniques she plans to employ for automated analysis of social behavior in mice. Her research and training plans are fully achievable within in the timeframe of the K99 award.

Dr. Padilla-Coreano's central goal is to use deep learning and unsupervised machine learning approaches for her behavioral data which will enable identification of mouse pose information and clustering into different behavioral motifs, respectively. This will be achieved by applying the DeepLabCut suite of tools for body-point tracking, a robust approach which is built upon the TensorFlow architecture for deep learning. Subsequently, the tracking data will be processed using unsupervised machine learning to cluster the data based on pose features into behavioral motifs. <u>Both of these goals do not require inventing new algorithms, but rather adapting and learning to use existing tools. Thus Dr. Padilla-Coreano's training goals are feasible for the timeframe.</u> My research group has extensive experience using deep learning approaches to study biological data, and we will ensure that Dr. Padilla-Coreano receives the support needed to develop technical expertise in this approach. Dr. Padilla-Coreano will attend our laboratory meetings and less formal team meetings to develop direct relationships with my lab to facilitate knowledge and expertise transfer. The Tye lab and my lab have active collaborations and Dr. Tye and I recently submitted a grant proposal together. In addition, I will have one-on-one meetings with Dr. Padilla-Coreano as she makes progress on her research for feedback and advice.

Dr. Padilla-Coreano currently has the necessary background in programming, including familiarity with Python, the language used for deep learning and machine learning tools. However, we have identified several key courses at UCSD (COGS 118A and COGS 18) for Dr. Padilla-Coreano to audit during her first year of the K99 training, which will provide formal instruction in Python and the background and theoretical underpinnings of machine learning approaches. This focused program of study will allow Dr. Padilla-Coreano to improve her skills early in her K99 training, and will not interfere with her research progress or transition to independence.

I am confident that Dr. Padilla-Coreano is an excellent applicant and that the K99 training goals will allow her to transition successfully to a competitive tenure-track position. Importantly, I have helped over 50 postdoctoral fellows transition to tenure-track positions and will provide my advice and guidance as Dr. Padilla-Coreano applies for job opportunities.

Sincerely,

Terrence Sejnowski

Terrence J. Sejnowski

Francis Crick Professor, Salk Institute
Distinguished Professor of Biology and Neurosciences, UCSD

October 3, 2019

To Whom It May Concern:

I am writing to strongly support Dr. Nancy Padilla-Coreano's K99/R00 'Pathway to Independence Award' application. As a member of her advisory committee, I will provide Dr. Padilla-Coreano with extensive guidance both during her training at the Salk Institute, and as she establishes her own research program. I believe that the success Dr. Padilla-Coreano has already had reflects her potential to become an outstanding independent researcher. Her proposed work investigating the neural circuits underlying social dominance behavior in mice is innovative and important. Moreover, Dr. Tye is an ideal primary mentor for this research program given her scientific background and outstanding record of excellence in research and mentoring.

I plan to work closely with Dr.Padilla-Coreano during her time at Salk. My lab is directly adjacent to the Tye lab; we share a common lunch room and lounge area, thus fostering constant communication between our lab members. Moreover, Dr. Padilla-Coreano will attend Callaway lab meetings. These interactions in lab meeting will help foster her framework for circuit dissection research and provide opportunities to learn how rabies tracing tools are best utilized. Importantly, I will have monthly in person meetings with Dr. Padilla-Coreano to discuss her research progress and challenges, and once she is applying for jobs to provide guidance to navigate the job market. I have ample experience helping postdocs transition to independence, as dozens of postdocs from my lab have transitioned to tenure-track faculty positions. An important part of the guidance for this stage will include a series of practice job talks and chalk talks, which will be attended by all members of Dr. Padilla-Coreano's advisory committee, where we will provide critical feedback on her presentation skills and help to ensure she is able to highlight the strengths of her research program.

As Dr. Padilla-Coreano transitions to her independent position and the R00 phase of her research plan, I will continue to be available for consultation and support with any challenges she may have. Training the next generation of scientific researchers is a top priority of the Salk Institute, and I will ensure that all the resources of our community are available to Dr. Padilla-Coreano for her training. I am fully committed to supporting Dr. Padilla-Coreano during her K99/R00 training and preparing her to lead a laboratory as a tenure-track faculty.

Sincerely,

Edward M. Callaway, Ph.D.
Vincent J. Coates Chair and Professor

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James P. Curley
Associate Professor
Psychology Department
University of Texas at Austin
curley@utexas.edu

September 30th 2019

I am writing to express my commitment to Dr. Nancy Padilla-Coreano in her K99/R00 award application in the role of a collaborator. As a collaborator I will provide both practical and conceptual guidance for the study of social dominance in female and male mice, and for the application of statistical methods for social behavior analysis. I have been a collaborator for Dr. Padilla-Coreano and her primary mentor, Dr. Kay Tye, for a couple of years. This ongoing collaboration has been fruitful, and we are presenting a poster in several conferences this year. I am confident that Dr. Padilla-Coreano's research and training plans are fully achievable within in the timeframe of the K99 award, and that this application will serve to successfully transition Dr. Padilla-Coreano to independence.

Dr. Padilla-Coreano aims to characterize social dominance behavior using a novel reward competition assay that she designed. She will use computer vision tools for tracking the pose of the mice and unsupervised machine learning for clustering the data into multiple behaviors. Once she has these behaviors labeled, we will collaborate to use social network analyses and other statistical approaches to quantify the behavioral differences in dominant vs subordinate mice. I have published numerous articles using social network analyses to study complex behaviors in mice, thus I have the expertise to provide guidance to Dr. Padilla-Coreano for her aims. To ensure communication, I will skype twice a month with Dr. Padilla-Coreano to discuss ongoing progress and challenges. Moreover, Dr. Padilla-Coreano and I will have two in person meetings per year, once during the Society for Social Neuroscience and she will visit my laboratory for the second meeting.

In summary, I am fully committed as a collaborator to Dr. Padilla-Coreano and we have all the tools to ensure the execution of the proposed research. Moreover, I am confident that Dr. Padilla-Coreano will successfully transition to a tenure-track position and become a prominent investigator in the field of social neuroscience.

Sincerely,

James Curley

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

The Salk Institute for Biological studies is an ideal environment for Dr. Padilla-Coreano's proposed research program and career development. During her training, Dr. Padilla-Coreano will benefit from the rich diversity of scientific research at the institute, and have support from a wide array of resources, training programs, and career development opportunities to launch her career as an independent and succesful investigator.

The institute was established in the 1963 by Dr. Jonas Salk, developer of the polio vaccine, with the goal of establishing a creative and collaborative environment for interdisciplinary research. The Salk Institute has maintained a tradition of excellence throughout its history, and is consistently ranked one of the top research institutions in the world. Today, the Salk Institute has 65 faculty members including three resident Nobel Laureates and four affiliated non-resident Nobel Laureates, and over 850 scientific staff. Dr. Padilla-Coreano's work has many potentially important implications for the study of neurological disorders, and as such is strongly aligned with the institute's mission to advance fundamental research in areas of the life sciences critical to human health. Neuroscience research is one of the strongest programs at the Salk institute, and Dr. Padilla-Coreano's colleagues will include renowned neuroscientists such Rusty Gage, Chuck Stevens, Thomas Albright, Sung Han, Saket Navlakha, as well her advisory committee members Terry Sejnowski and Ed Callaway. These faculty members have expertise in key areas related to Dr. Padilla-Coreano's study including systems neuroscience and computational neuroscience, and will provide support and mentorship for Dr. Padilla-Coreano during her training.

Within the Salk Institute, Dr. Padilla-Coreano and her primary supervisor, Dr. Kay Tye, are affiliated with the Systems Neuroscience Laboratory (SNL) and will have full access to state-of-the-art core facilities including the AALAC accredited facilities in the Animal Resources Department, the Viral Vector Core, the Behavior Testing Core, the Biophotonics Core Facility and the Crick-Jacobs Center for Theoretical and Computational Biology. These facilities are a major strength of the institute and will provide expert technical support and guidance for Dr. Padilla-Coreano in her research. The Tye lab occupies an expansive and newly-renovated space which was designed in consultation with members of the lab including Dr. Padilla-Coreano, and is ideally configured for all research aims in the K99 period of her award.

To ensure that Dr. Padilla-Coreano develops the skills needed to succeed as an independent researcher, The Salk Institute also offers many resources for professional development. These include meetings and seminars organized by the Salk Society of Research Fellows (SRF) such as the 'Salk Featured Fellows' program, a monthly session which allows postdoctoral fellows to present their research, and the 'Coffee with a PI' series where small groups of fellows spend the morning with a Salk faculty member to foster collaboration and establish new sources of mentorship for participating fellows. The SRF also provides competitive travel awards for postdoctoral fellows and leadership opportunities for fellows to participate as a rotating Committee Chair. The Salk Institute is also partnered with neighboring institutions through the Torrey Pines Training Consortium (TPTC), which has representatives from the Salk Institute, the University of California San Diego (UCSD) and the Scripps research institute. The TPTC provides many resources for postdoctoral fellow training including the Academic Leadership Symposium, a two-day course on laboratory management, 'Funding Fest', a month-long event with multiple workshops and seminars covering different facets of scientific grant writing, and the 'Career Building' seminar series. To increase her exposure to diverse scientific research, Dr. Padilla-Coreano will also be able to attend the First Friday symposium series, the weekly Salk Institute seminar series, and, through her lab's affiliation with UCSD, seminars and workshops hosted by the Institute for Neural Computation (INC) and Center for Neural Circuits and Behavior (CNCB).

Altogether, Dr. Padilla-Coreano will have access to the full resources of the Salk research community to provide support for her research and career development. The many training programs offered by our institute will ensure that Dr. Padilla-Coreano with the skills needed for her transition to independence, to obtain a tenure-track position and to lead a successful research program in the R00 phase of the award.

This statement was prepared in collaboration with the Salk Postdoctoral Office.

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October 8, 2019

Institutional Commitment to Candidate's Research Career Development

The Systems Neuroscience Laboratory (SNL) at the Salk Institute for Biological Studies is in full support of Dr. Nancy Padilla Coreano's application for the BRAIN Initiative K99/R00 Pathway to Independence Award.

The Salk Institute is dedicated to providing postdoctoral training associated with a variety of professional development opportunities outside of the laboratory setting to promote successful transitions into independent research positions. Career development of young scientists is an important part of the Institute's educational mission. The Salk Institute will therefore stand behind the Faculty mentoring team (Dr. Kay Tye, Dr. Terry Sejnowski and Dr. Ed Callaway) in their efforts to help Dr. Nancy Padilla Coreano develop towards a career as a productive, independent investigator.

The Systems Neurobiology Laboratory (SNL) at The Salk Institute agrees to provide adequate time and support for the candidate to devote full-time to research career development for the entire period of the proposed award. SNL will provide Dr. Padilla the equipment, facilities and resources necessary for a structured career development, and The Salk Institute is committed to her development and advancement, independent of the receipt of this career award. Prior to securing independent appointments elsewhere, with support from the mentor and approval of the Salk Appointments Committee, postdoctoral trainees have the option to become Staff Scientists (a non-tenure-track position supporting independent research and encouraging applications for independent funding).

a. The Salk Institute is an academic research organization. Dr. Padilla will have no responsibilities other than her research project in SNL. She will devote 100% of her time to research career development during the award period.

b. Dr. Padilla has a full-time appointment as a Postdoctoral Fellow.

c. Dr. Padilla currently spends 100% of her time devoted to research; that is not expected to change should this proposal be funded.

d. Dr. Padilla has adequate desk and laboratory space, including access to equipment and facilities as detailed in the Resources and Equipment sections of this proposal.

e. Consistent with the career development plan, the mentorship team (Drs. Tye, Sejnowski and Callaway) and sponsor (Dr. Tye) will provide their full support and adequate time to Dr. Padilla as detailed in the Sponsor Statement.

Sincerely,

Thomas D. Albright
Professor and Head
Systems Neurobiology Laboratory

Kim E. Witmer
Senior Vice President
Finance and Administration

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

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	
Does the proposed research involve human specimens and/or data	❍ Yes	● No

Other Requested information

VERTEBRATE ANIMALS

Vertebrate Animals Section (VAS)

1. Description of Procedures

Surgical Procedures: During surgery every effort is made to minimize the discomfort of the mice. Equal numbers of C57BL6/J background female and male mice between 8-10 weeks old will be used for surgeries. Prior to surgery Buprenorphine at (0.05 mg/kg) will be administered via subcutaneous injection in addition to the local anesthetic lidocaine (2%) at the site of incision. Additionally, non-puncturing stereotaxic ear bars are coated with lidocaine gel prior to securement to alleviate discomfort. Rats will be anesthetized with inhalation of isoflurane/oxygen mixture (5% induction, 1-5% maintenance). Anesthesia levels will be frequently (every 3 min) monitored by absence of palpebral reflex (eye blink), by the absence of foot withdrawal following pressure to the footpad, or by any other overt signs of response to physical stimuli. The level of anesthesia will be adjusted to maintain adequate anesthesia during surgery. The eyes will be protected by application of sterile ophthalmic grade ointment. The skin will be re-sealed using surgical sterile nylon sutures. The animal will then be removed and placed near a heating blanket or a heat lamp positioned in a way such that movement away from the heat source will be possible once animals are ambulatory. Fresh wet food will be provided every 8-12 hours, to allow the animal easy access to both nutrition and hydration. Following surgery, Buprenorphine at 0.05 mg/kg is administered by subcutaneous injection every 8-12 hours for the first 48 hours post-surgery and continued if signs of pain persist. Animals exhibiting morbidity cannot be used in our studies and will be euthanized. If the implant is lost or loosened, the animal will also be euthanized immediately.

Behavioral Tests: After 6 weeks of surgery behavioral experiments (tube test, open field, food assay in home cage) will be done between 9am-6pm and during the reward competition assay and effort based-T maze mice will be food restricted to no more than 85% of their body weight in accordance with the IACUC protocol.

Animals to be used: In the present study we will use C57BL6/J background and two lines of transgenic mice which are maintained on a C57BL6/J background (see also 'Authentication of Reagents' document):
'vGAT::Cre' mice: Vgat-ires-cre knock-in; RRID: IMSR_JAX: 028862
'vGLUT::Cre' mice: Vglut2-ires-cre knock-in RRID: IMSR_JAX: 028863
Sex differences: In the present study, equal numbers of male and female mice will be used in all experiments in accordance with NIH statement on 'Consideration of Sex as a Biological Variable'. In all experiments, data from males and females will be examined to determine if sex differences exist in behavioral or electrophysiological data recorded. If no differences exist, groups will be pooled together if appropriate, though disaggregated data for each sex will still be reported in our publication of the data or available upon request by other researchers.
Attrition rate and group size: Due to the difficulty of targeting deep brain structures such as the lateral hypothalamus (LH) we anticipate that data from some of animals will be excluded due to our strict criteria for acceptable accuracy of stereotaxic procedures. In addition, there are some rare cases in which cages do not for stable dominance hierarchies in which case these animals would be excluded from the study. Consequently, we estimate the attrition rate for these experiments at 25%. In all experiments, an absolute minimum of subjects will be used to achieve acceptable statistical power. Based on power calculations, each comparison will require a final n=6 animals. Assumptions: power = 0.9; alpha = 0.05; two-tailed and an expected difference 50% greater than the observed standard deviation. Therefore, I will initially aim for 12 animals (6 male, 6 female) per proposed experimental group to allow for sex differences detection.

Mentored Phase Experiments (Aims 1-3):
Experiment 1.1: 1 group (WT C57BL6/J) x 12 mice per group............12
Experiment 2.1. 1 group (WT C57BL6/J) x 12 per group............12
Experiment 2.2: 2 groups (ChR2 and eYFP) x 12 mice per group............24
Experiment 2.3: 4 groups (vGAT::Cre/Chrimson, vGAT::Cre/mCherry, vGLUT::Cre/Chrimson, vGLUT::Cre/mCherry) x 12 mice per group............48
Experiment 3.1: 2 groups (NpHR and eYFP) x 12 mice per group............24
Experiment 3.2: 2 groups (ChR2 and eYFP) x 12 mice per group............24
Attrition (25%)............36
Subtotal 180

Independent Phase Experiments (Aim 4):
Experiment 4.1: 2 groups (NpHR and eYFP) x 12 mice per group....................24
Experiment 4.2: 2 groups (NpHR and eYFP) x 12 mice per group....................24
Experiment 4.3: 2 groups (NpHR and eYFP) x 12 mice per group....................24
Attrition (25%) 18
Subtotal 90

Grand total: 270 mice

2. Justifications
Mice will be used as an experimental model as they provide a high-throughput system for rapid optimization across many iterations to offer insight into the neural circuitry of the brain. These studies cannot be done in humans for ethical reasons and a suitable computer model is not available. The methods used are consistent with the accepted practices within the field and no appropriate alternatives are available. The specific scientific questions posed here cannot be answered from any other informational source(s). For instance, computational modeling does not mimic the complexity of the vertebrate brain, thus the use of vertebrate animals is necessary. Additionally, since both cell and tissue cultures do not have the synaptic connections, plasticity or physiology of the whole animal, they also cannot be used to answer our questions. Finally, we utilized a within-subject design for many of the proposed experiments, which will allow us to minimize the number of animals used by completing multiple measures within each animal.

3. Minimization of Pain and Distress
During all procedures, every effort will be taken to minimize pain and discomfort in animals. *Survival surgical procedures:* All surgical procedures will be conducted in accordance with IACUC standards. Mice 6-8 weeks of age weighing at least 25g will be put under general anaesthesia with isofluorane (5% induction, 1-3% maintenance, in O_2), with depth of surgical anesthesia verified by absence of eye blink and foot withdrawal responses to appropriate stimulation. Mice will also be administered slow-release burprenorphine (0.05 mg/kg) and subcutaneous lidocaine injection (2%) at the site of surgical incision. All surgical tools and the entire surgical area will be sterilized and aseptic techniques used throughout the procedure. During surgery, sterile opthalmic lubricant will be applied to prevent dehydration of eyes, and temperature will be maintained at 37°C via a heating blanket and rectal thermometer. After viral injection or surgical implantation, skin will be sealed with sterile nylon sutures. In the post-surgical period, mice will be placed alone in a clean cage half-over a heating pad with ample water and soft food available. Mice will be monitored daily for 4 days after surgery, and additional Buprenorphine SR will administered during the first two days post-surgery and as needed thereafter. *Behavioral Experiments:* During the reward conditioning and the Tmaze effort experiment mice will be food restricted. Body weights and general health of mice will be recorded each day, and mice will be provided 4.0 g of food per day (supplemented by task rewards), with more as needed to maintain body weight. If animals show any signs of distress, or body weight falls below 15% of starting weight, they will be removed from the experiment. Mice will be given ad libitum access to water, and housed in cages with dry bedding (changed bi-weekly), and ample materials for nest construction. Food restriction experiments will be registered in advance and monitored by staff veterinarians.

4. Method of Euthanasia

The animals are sacrificed at the necessary time points for analysis. Specifically, a lethal dose of sodium pentobarbital (100 mg/kg) administered IP followed by pericardial perfusion. Both are preferred methods in accordance with the AVMA guidelines and provide a rapid and painless loss of consciousness and subsequent death. For analysis by confocal microscopy, the brains are fixed, sliced, stained, and imaged using a confocal microscope.

SELECT AGENT RESEARCH

No select agents (hazardous biological agents or toxins) will be used in the present research proposal.

For this project viral vectors will be used to express the genes of interest and relevant fluorophores in neurons. Viral vectors are from commercial sources and all tittering is done by the manufacturer under their standard guidelines. Our vectors are considered Biohazard Level 2 (BL2). This is because they are non-infectious and cannot replicate, and the only protein which they encode is the transgene of interest. Although precautions regarding exposure will be taken while handling the AAV5, modified rabies and CAV-2 viruses necessary for this project, these strains do not infect humans. These viral strains are nonpathogenic, and share only a portion of their genome with the wild-type virus on which they are based. Recombinant strains do not "infect", but rather need to be transduced into the neuron under the promoter of interest, where they do not replicate but spread by neuronal transport within the projections of that neuron. All viruses are deemed replication-defective as they are not just mutated, but are actually missing a large portion of their genome that encodes the viral gene products necessary for replication. This allows for expression in the cells, but no progeny virus can be produced. All viruses are pseudotypes specific for expression of neuronal tissue, and will only express under the control of a neuronal promoter. In addition, the transgenes of interest do not encode for toxins or oncogenes. These viral vectors will be used to deliver opsin and fluorophore-expressing constructs to targeted neuronal populations in experimental mice, but a majority of the viral structural genes have been removed rendering the virus unable to replicate and safe for use. These viral vectors will be supplied by the University of North Carolina (UNC) viral core.

AAV vector MSDS: https://www.med.unc.edu/genetherapy/vectorcore/files/2018/07/unc-vc-raav-msds.pdf

Additionally, all standard precautions will be taken during stereotaxic surgeries using these viral vectors to prevent any contact with humans. Viral vectors will be stored in a dedicated -80°C freezer, and small volumes of virus (<1 uL) will be transferred in disposable pipettes for loading into micropipettes for injections. All standard personal protective equipment for surgeries (mask, gloves, lab coat and safety glasses) will be used when handling these reagents.

RESOURCE SHARING

Data Sharing Plan

Upon publication of the manuscripts based on the proposed research study, all data will be available upon request to members of the scientific community in accordance with the NIH Data Sharing Policy and other NIH guidelines. We will also provide any relevant code, protocols and hardware specifications for custom-built components to other research groups upon request.

Sharing of Model Organisms for Biomedical Research

We do not have plans to generate any novel transgenic mouse lines as part of this research program.
All mouse lines used in the proposed experiments will be sourced from the Mutant Mouse Resource & Research Centers (MMRRC) and The Jackson Laboratory (JAX), and are available to other researchers through these organizations in compliance with NIH Policy on Sharing of Model Organisms for Biomedical Research.

The Salk Institute is committed to ensuring that biomedical research resources, including model organisms, developed with NIH funding are made readily available to the research community in compliance with the NIH Grants Policy Statement, NIH Research Tools Policy and NIH Policy on Sharing of Model Organisms for Biomedical Research. The Salk Institute has a streamlined Material Transfer Agreement (MTA) process for transferring research resources freely to the research community for non-commercial research purposes. Salk Institute investigators simply include the Institute's standard MTA with the shipment of research resources and signatures are obtained after the transfer is made. The Salk Institute's standard one page MTA is less restrictive and much shorter than the Uniform Biological Material Transfer Agreement (UBMTA). The Salk Institute avoids signing MTAs from outside parties that include royalty or product reach-throughs for materials that will be used in NIH funded projects so that access to subsequent research resources arising from such projects will not be restricted.

Genomic Data Sharing

No new large-scale genomic data sets will be produced in the proposed experiments. If any additional data is produced from examining gene expression in target neuronal populations (i.e. RNAseq) it will be included in publication of our research with additional data available upon request as outlined above, however we are not currently planning any such experiments.