This document is provided as a sample application. Some pages and text have been redacted.

PI: **Aronov, Dmitriy**	Title: Using a specialized behavior to study the neural mechanisms of episodic memory	
Received: 08/26/2019	Opportunity: RFA-RM-19-006 Clinical Trial:Optional	Council: 05/2020
Competition ID: FORMS-E	FOA Title: NIH Directors New Innovator Award Program (DP2 Clinical Trial Optional)	
1DP2AG071918-01	Dual: RM,OD	Accession Number: 4341003
IPF: 1833205	Organization: COLUMBIA UNIVERSITY HEALTH SCIENCES	
Former Number: 1DP2OD029406-01	Department: Neuroscience	
IRG/SRG: ZRG1 MOSS-R (70)R	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 1,500,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Y Early Stage Investigator: Y
Senior/Key Personnel:	*Organization:*	*Role Category:*
Dmitriy Aronov	The Trustees of Columbia University in the City of New York	PD/PI

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number
7 NS; 8 HIB

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: The Trustees of Columbia University in the City of New York
Department: Zuckerman Institute
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Rudina Middle Name: Last Name*: Odeh-Ramadan Suffix:
Position/Title: Vice President for Research Administration
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

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

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Using a specialized behavior to study the neural mechanisms of episodic memory

12. PROPOSED PROJECT
Start Date*: 08/15/2020
Ending Date*: 06/30/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
NY-013

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Dmitriy Middle Name: Last Name*: Aronov Suffix:

Position/Title: Assistant Professor

Organization Name*: The Trustees of Columbia University in the City of New York

Department: [redacted]

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$1,500,000.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$1,500,000.00
d. Estimated Program Income*	$0.00

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

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

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Madhavi Middle Name: Last Name*: Nambiar Suffix: Ph.D

Position/Title*: Senior Project Officer

Organization Name*: The Trustees of Columbia University in the City of New York

Department: [redacted]

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

Signature of Authorized Representative*

Madhavi Nambiar

Date Signed*

08/26/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

0MB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: The Trustees of Columbia University in the City of New York

Duns Number: [redacted]
Street1•: [redacted]
Street2: [redacted]
City•: [redacted]
County:
State•: [redacted]
Province:
Country•: [redacted]
Zip / Postal Code•: [redacted]
Project/Performance Site Congressional District-: NY-013

Additional Location(s) File Name:

Funding Opportunity Number: RFA-RM-19-006. Received Date: 2019-08-26T11:40:48.000-04:00

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:

Animal Welfare Assurance Number [redacted]

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Summary.pdf
8. Project Narrative*	Project_narrative.pdf
9. Bibliography & References Cited	
10.Facilities & Other Resources	Facilities_and_Other_Resources.pdf
11.Equipment	

Funding Opportunity Number: RFA-RM-19-006. Received Date: 2019-08-26T11:40:48.000-04:00

Project summary

Throughout the day, the brain captures snapshots of distinct, instantaneous experiences, forming episodic memories that often last a lifetime. These types of single-shot memories require the hippocampus and the entorhinal cortex – a circuit collectively called the hippocampal formation. Disruptions of this brain region are involved in several devastating memory disorders, including Alzheimer's disease. In spite of extensive study, we still lack basic understanding of how activity in the hippocampus implements memory functions.

Neuroscience has amassed impressive knowledge about neural firing patterns in the hippocampal formation, including those of place cells and grid cells. Yet, these cells are best understood in static conditions, once an animal has learned an environment and has been extensively trained on a behavioral task. We lack a clear connection between hippocampal activity and dynamic processes of memory formation and recall. How does hippocampal activity change when a new memory is formed? How are these firing patterns interpreted by other brain regions when a memory is recalled? These questions are challenging to address because the hippocampal formation is anatomically extremely complex, and because episodic memory-guided behaviors are particularly difficult to study in standard laboratory model organisms.

In this project, we seek to overcome major challenges to hippocampal research by using a unique model organism that is an extreme memory specialist – the chickadee. These birds cache thousands of food items at scattered, hidden locations in their environment and use memory to retrieve their caches later in time. Their behavior is readily produced in the lab and contains well-defined moments of memory formation (caching) and recall (cache retrieval). The repeatable and streamlined structure of food caching provides an opportunity to study neural activity underlying these memory processes. Cache memory requires the avian hippocampal formation, which is embryologically homologous to its mammalian counterpart and shares similar circuit organization. However, the avian hippocampus is anatomically simpler and has a small number of well-defined, compact, and thus easily targetable inputs and outputs.

The proposed project will obtain recordings of the hippocampus while chickadees are actively caching and retrieving food. This will allow us to relate hippocampal activity to discrete memory processes and to obtain an interpretable neural signature of episodic memories. By leveraging chickadee anatomy, this project will also determine what information is conveyed by hippocampal outputs to identified targets in the brain during memory recall. The ultimate goal is to obtain a complete circuit-level understanding of episodic memory. Because of the close correspondence between our system and the mammalian hippocampus, these findings will inform other fields and will generalize to hippocampal systems in other organisms that use memory, including humans.

Project narrative

The proposed project aims to acquire a neural circuit-level understanding of how memories of everyday events, known as “episodic” memories, are formed and recalled in the brain. This function relies on the hippocampal formation – a brain region whose disruption results in a variety of memory disorders like Alzheimer’s disease. Our approach is to use the highly specialized and complex memory behavior of food caching birds to understand the relationship between memories and neural activity in the hippocampal formation.

Facilities and Other Resources

Laboratory: Dr. Aronov's laboratory is housed in approximately 1500 sq. ft. of contiguous space at Columbia University's brand-new Jerome L. Greene Science Center. The laboratory contains 1) wet-lab space with 6 benches for electronics, small-device construction, and histological tissue processing, 2) a chemical alcove that includes a chemical fume hood, 3) desks for up to 8 students or postdocs, 4) three separate sound-isolated rooms for behavioral experiments, electrophysiological recordings and imaging, 5) an enclosed surgery suite outfitted with two bird stereotaxic devices, 6) an equipment workshop 7) a booth for epifluorescence microscopy and 8) a vivarium satellite room for housing birds for the duration of an experiment (additional birds are housed in the main vivarium space; see below)

Office: Dr. Aronov's 130 sq. ft. office opens directly into the wet-lab space.

Institutional support: In addition to the laboratory space, Dr. Aronov has been provided with a generous startup package, including full salary support for three years. No teaching is required, although small graduate classes can be taught on a voluntary basis. Dr. Aronov is appointed with the Department of Neuroscience and is a member of the Zuckerman Mind Brain Behavior Institute. He is also a member of the Columbia Doctoral Program in Neurobiology and Behavior, which attracts excellent graduate students. Students are fully funded by the program for the first year.

Opportunities for interaction and collaboration: The Zuckerman Mind Brain Behavior Institute contains 56 labs in neuroscience and related disciplines, housed under one roof in the newly occupied building. At least 13 of these labs study the hippocampus, providing ample opportunities for intellectual and logistical interactions. These labs include those of [redacted]. Many additional labs are involved in small-animal physiology, including investigators working on birds: [redacted]. Another major resource in the building is the Center for Theoretical Neuroscience, which is a world-class center for researchers working on data analysis techniques and computational modeling. This center includes [redacted], both of whom have ongoing collaborations with the Aronov lab.

Clinical: Not applicable.

Animals: Animals are kept in the same building as the Aronov lab, in a state-of-the-art AAALAC-accredited vivarium facility. The vivarium includes an aviary with two rooms dedicated to Dr. Aronov's birds. New cages were purchased for the aviary on Dr. Aronov's behalf. To minimize transport of animals, the vivarium also includes a satellite room located directly adjacent to the Aronov lab, housing birds for the duration of an experiment.

Computer: Each behavioral and recording setup necessary for the project will include 1-2 computers. Additionally, each lab member will have a computer for data analysis. These computers have already been purchased.

Other: Dr. Aronov and his lab members have unrestricted access to several staffed core facilities in the building. Facilities useful for the proposed project include 1) the Advanced Instrumentation Group, which collaborates with labs to design custom mechanical and electronic devices, 2) the Cellular Imaging facility for confocal and automated epifluorescence tissue microscopy, 3) the Virology Core for custom recombinant virus design and 4) the Research Computing Group, which provides assistance with parallel computing and data storage.

Biohazards: The adeno-associated viral (AAV) particles to be used are deemed by Columbia University's Health & Safety Department as low-risk viral vectors under Biosafety Level 1 (BSL-1). These viruses are non-pathogenic and replication incompetent, and therefore cannot reproduce inside the animal. Not only do AAVs require genetic material from the adenovirus in order to properly reproduce, certain genes from the AAV itself have also been removed, rendering it quite safe to use. Standard aseptic technique, gloves, lab coats and eye protection will be used. A benchtop sink is available for hand washing, and everything is wiped with 10% bleach for inactivation as an extra precaution.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Dmitriy Middle Name Last Name*: Aronov Suffix:

Position/Title*: Assistant Professor
Organization Name*: The Trustees of Columbia University in the City of New York
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Fax Number:

E-Mail*:

Credential, e.g., agency login:

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

Degree Type: PHD,BS Degree Year: 2010,2005

Attach Biographical Sketch*: File Name: Biosketch_for_DP2_2.pdf

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

Tracking Number: GRANT12924272 Funding Opportunity Number: RFA-RM-19-006 . Received Date: 2019-08-26T11:40:48.000-04:00

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: Dmitriy Aronov

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Columbia University, New York, NY	B.S.	06/2005	Applied Mathematics
Massachusetts Inst. of Tech. (MIT), Cambridge, MA	Ph.D.	09/2010	Neuroscience
Princeton University, Princeton, NJ	Postdoctoral	12/2016	Neuroscience

A. Personal Statement

Memory of events, known as episodic memory, is the foundation of everyday experience that is disrupted in a variety of devastating memory disorders. My central goal is to understand the neural dynamics that underlie this process and the circuit mechanisms by which episodic memories are recalled later in time. Achieving this goal will require understanding the relationship between specific memories and activity in the hippocampal formation – the neural circuit critical to memory functions. My lab addresses this question by studying the hippocampal formation in food-caching birds – extreme specialists that hide thousands of food items in their environment and use hippocampal memory to find these caches. We pioneered the use of this model organism in systems neuroscience. Over the past two years, we have engineered a variety of tools that make food-caching birds compatible with a wide range of modern neuroscience methods, including high-throughput behavioral training and analysis of natural behavior in lab conditions, high-density electrophysiology, functional calcium imaging, pharmacology, and optogenetics. We also use advanced quantitative techniques for data analysis and modeling and collaborate extensively with computational and theoretical neuroscientists at Columbia University. My prior work is a unique combination of extensive work on small songbirds and on the mammalian hippocampal formation. These areas of expertise are remarkably synergistic for the proposed project.

B. Positions and Honors

Positions and employment

1999 – 2001 Research Student, Weill Cornell Medical College. PI: [redacted].
2001 – 2005 Research Student, Columbia University. PI: [redacted].
2005 – 2010 Graduate Student, Herz Foundation Fellow, MIT. PI: [redacted].
2010 – 2016 Postdoctoral Fellow, Helen Hay Whitney Fellow, Princeton University. PI: [redacted].
2017 – present Assistant Professor of Neuroscience, Columbia University.

Honors

2001 National finalist, Intel Science Talent Search
2001 – 2005 C. P. Davis Scholarship, Columbia University
2005 Magna cum laude, Columbia University
2005 – 2006 Presidential Fellowship, MIT

2006 – 2010 Hertz Foundation Fellowship (Silvio Micali fellow)
2006 Agnus MacDonald Award for Excellence in Undergraduate Teaching, MIT
2008 Walle Nauta Award for Continuing Dedication to Teaching, MIT
2012 – 2015 Helen Hay Whitney Foundation Fellowship (Howard Hughes Medical Institute fellow)
2015 – present K99/R00 Pathway to Independence Award (NINDS)
2018 – present Beckman Young Investigator, Arnold and Mabel Beckman Foundation
2019 – present Robertson Investigator, New York Stem Cell Foundation

Professional memberships

2006 – present Member, Society for Neuroscience

C. Contributions to Science

1. Understanding the general nature of hippocampal representations. My recent work addressed the nature of neural representations in the hippocampus and the entorhinal cortex. Studies have examined these brain regions during navigation and have found a great variety of activity patterns that are correlated to spatial variables (e.g. location). These specialized patterns have been difficult to reconcile with the general role of the hippocampal circuit in memory and cognition. I designed a joystick-based task in which rats manipulated a non-spatial variable, sound frequency. I found that the same neurons that are canonically thought to encode spatial location (e.g. place and grid cells) exhibited activity correlated to frequency, suggesting a more general role for these activity patterns in arbitrary behaviors. I also designed and implemented a novel virtual reality system for rats, in which animals are sufficiently unconstrained to perform 2D navigation behaviors. I found that the major activity patterns in the hippocampal/entorhinal circuit are engaged in virtual reality when only visual and rotational cues are provided to the animal. This system is a technological advance that will allow recording hippocampal/entorhinal activity during well-controlled, temporally precise environmental manipulations. My published contribution to the hippocampus field also includes developing tools for analyzing oscillations and other activity patterns in the developing hippocampus.

[1] **Aronov, D.**, Nevers, R., and Tank, D.W. (2017) Mapping of a non-spatial dimension by the hippocampal–entorhinal circuit. Nature 543(7647): 719-22. PMCID5492514.

[2] **Aronov, D.** and Tank, D.W. (2014) Engagement of the neural circuits underlying 2D spatial navigation in a rodent virtual reality system. Neuron 84(2): 442-56. PMCID4454359.

[3] Crepel, V., **Aronov, D.**, Represa, A., Ben-Ari, Y., and Cossart R. (2007) A parturition-associated nonsynaptic coherent activity pattern in the developing hippocampus. Neuron 54(1):105-20.

2. Dissecting the pathways for early motor control in the song system. Part of my earlier work was dedicated to the dissection of pathways for early motor control in the zebra finch song system. Young finches produce noisy babbling called subsong. Via trial-and-error learning, these early vocalizations are gradually transitioned into stereotyped adult singing. It was not known what changes to neural dynamics underlie this transition. Using single-unit neural recordings and various circuit manipulation techniques, I identified a forebrain pathway that produces babbling vocalizations. Surprisingly, this pathway was distinct from the one that produces singing in adulthood. In a follow-up study, I determined that the two pathways – for juvenile and for adult singing – were specialized for producing fundamentally different types of neural dynamics: temporally random vs. temporally stereotyped. This provided the field with a novel way to think about the specialization of distinct brain regions in the song system and a new model for how stereotyped motor patterns arise in development.

[4] **Aronov, D.**, Veit, L., Goldberg, J.H., and Fee, M.S. (2011) Two distinct modes of forebrain circuit dynamics underlie temporal patterning in the vocalizations of young songbirds. J Neurosci 31(45):16353-68. PMCID3241969.

[5] Ölveczky, B., Otchy, T., Goldberg, J.H., **Aronov, D.**, and Fee, M.S. (2011) Changes in the neural control of a complex motor sequence during learning. J Neurophysiol 197(1): 32-47. PMCID3129720.

[6] **Aronov, D.**, Andalman, A.S., Fee, M.S. (2008) A specialized forebrain circuit for vocal babbling in the juvenile songbird. Science 320(5876):630-4.

3. Developing tools for work in small songbirds. Neuroscience experiments on freely behaving animals are particularly difficult on small songbirds. Another part of my graduate work was therefore dedicated to the engineering of novel tools for circuit recordings and manipulations. These have included devices for electrophysiology that I improved and miniaturized, as well as entirely novel devices for pharmacology, Peltier cooling, brain temperature recordings, and respiratory measurements.

[7] Danish, H.H., **Aronov, D.**, and Fee, M.S. (2017) Rhythmic syllable-related activity in a songbird motor thalamic nucleus necessary for learned vocalizations. PLoS One 12(6): e0169568.

[8] Veit, L., **Aronov, D.**, and Fee, M.S. (2011) Learning to breathe and sing: development of respiratory-vocal coordination in young songbirds. J Neurophysiol 106(4):1747-65. PMCID3191841.

[9] **Aronov, D.** and Fee, M.S. (2012) Natural changes in brain temperature underlie variations in song tempo during a mating behavior. PLoS One 7(10):e47856. PMCID3480430.

[10] **Aronov, D.** and Fee, M.S. (2011) Analyzing the dynamics of brain circuits with temperature: design and implementation of a miniature thermoelectric device. J Neurosci Methods 197(1): 32-47. PMCID3070058.

4. Developing analytical tools for the analysis of spike trains. Analyses of neuronal spike sequences often don't take into account the timing of individual spikes. Exact timing, however, matters for processes that are sensitive to the coincidence of spikes from different neurons. Some of my early work dealt with developing efficient 'metric-space' methods for characterizing the temporal structure of neural responses. These methods measure the similarity of two spike trains by quantifying similarities in overall firing rates as well as temporal coincidences of individual spikes. I developed efficient computer algorithms and mathematically characterized these methods. I also used these methods to identify temporal components of neural responses in the primate visual cortex that are informative about particular features of the visual stimulus.

[11] **Aronov, D.** and Victor, J.D. (2004) Non-Euclidean properties of spike train metric spaces. Phys Rev E 69(6 Pt 1): 061905. PMCID2911631.

[12] **Aronov, D.**, Reich, D.S., Mechler, F., and Victor, J.D. (2003) Neural coding of spatial phase in V1 of the macaque monkey. J Neurophysiol 89(6): 3304-27.

[13] **Aronov, D.** (2003) Fast algorithm for the metric-space analysis of simultaneous responses of multiple single neurons. J Neurosci Methods 124(2): 175-9.

5. Developing analytical tools for the analysis of calcium imaging data. Calcium imaging is a dominant technique for recording activity from large neuronal populations. However, identifying individual neurons in a movie and extracting neural signals are extremely time consuming processes. That is generally fine for post-experiment analysis, but is inadequate if signal extraction needs to be performed during the experiment. My other early work was concerned with developing algorithms for fast analysis of calcium imaging data obtained from brain slices. These algorithms were precise enough to identify neurons that participated in particular patterns of synchronous network activity and were used to target these neurons for subsequent intracellular recordings. My analysis of calcium activity also extended to the modeling of calcium dynamics in dendrites.

[14] Ikegaya, Y., Aaron, G., Cossart, R., **Aronov, D.**, Lampl, I., Ferster, D., and Yuste, R. (2004) Synfire chains and cortical songs: elastic temporal modules of cortical activity. Science 304(5670): 559-64.

[15] Goldberg, J.H., Tamas, G., **Aronov, D.**, and Yuste, R. (2003) Calcium microdomains in aspiny dendrites. Neuron 40(4): 807-21.

[16] Cossart, R., **Aronov, D.** and Yuste, R. (2003) Attractor dynamics of network UP states in the neocortex. Nature 423(6937): 283-8.

[17] Mao, B.Q., Hamzei-Sichani, F., **Aronov, D.**, Froemke, R.C. and Yuste, R. (2001) Dynamics of spontaneous activity in neocortical slices. Neuron 32(5): 883-98.

D. Additional Information: Research Support

Ongoing research

R00 NS093071 Aronov (PI) 05/01/2017 – 04/30/2020
Mechanisms of spatial memory during navigation in virtual reality

Robertson Investigator Aronov (PI) 01/01/2019 – 12/31/2023
New York Stem Cell Foundation
Neural circuit mechanisms of episodic memory

Beckman Young Investigator Aronov (PI) 09/01/2018 – 08/31/2022
Arnold and Mabel Beckman Foundation
Neural mechanisms of episodic memory

Completed research

K99 NS093071 Aronov (PI) 07/01/2015 – 12/31/2016
Mechanisms of spatial memory during navigation in virtual reality

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)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. 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:

Tracking Number: GRANT12924272 Funding Opportunity Number: RFA-RM-19-006 . Received Date: 2019-08-26T11:40:48.000-04:00

PHS 398 Research Plan

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

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	
3. Research Strategy*	Research_strategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_2.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	
11. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Biological_and_Chemical.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12924272
Funding Opportunity Number: RFA-RM-19-006. Received Date: 2019-08-26T11:40:48.000-04:00

PROJECT SCIENCE AREAS

7. NS, 8. HIB

PROJECT DESCRIPTION

Using a specialized behavior to study the neural mechanisms of episodic memory

On a February morning in 2013, a meteor exploded over a city in Siberia. Without ever repeating again, this event triggered lifelong "episodic" memories[1] in the thousands of brains that witnessed it. Accounts of this event are quite remarkable: even though the explosion only lasted a few seconds, most eyewitnesses recall vast amounts of diverse information about it, like sensory stimuli and co-occurring emotions. How does the brain use single-shot, brief events to form permanent representations of experience? How does it use these representations later in life to influence decisions and behavior? To form and recall episodic memories, the brain requires the hippocampus and the entorhinal cortex – a neural circuit collectively called the hippocampal formation[2–4]. In spite of extensive study, we still lack basic understanding of how activity in the hippocampal formation encodes individual memories.

I suggest using a unique model to study the hippocampal formation and the mechanisms of episodic memory. This model is the chickadee – a bird that naturally caches large amounts of food in its environment and remembers an impressive number of individual cache locations. My proposal is a sharp and innovative departure from the rest of the field, as well as from my own prior career trajectory. In this essay, I hope to relay my conviction that this is a promising fresh approach to some of the toughest challenges in neuroscience.

The hippocampal formation is one of the most intensely studied regions in the brain. Half a century of study has created an impressive account of neural activity in this region – an effort that culminated with the 2014 Nobel Prize in Physiology or Medicine. Activity of many neurons in the hippocampal formation, like place cells and grid cells, is strongly correlated to the animal's location[5,6]. A body of recent work[7–9] (including my own[10]) shows that these neurons also represent other variables relevant to the animal. Yet, our understanding of the hippocampus is plagued by the lack of a clear connection between these activity patterns and memory. How does a pattern of hippocampal activity correspond to a specific episodic memory? How is this pattern read out by other brain regions during memory recall?

These questions have critical consequences for human health. Disruptions of the hippocampal circuit lead to a number of devastating memory disorders, including Alzheimer's disease[4,11]. Neural interventions are becoming effective in other regions at treating neurological and even psychiatric diseases[12]. Yet, they have noticeably lagged in the hippocampal system. In a poignant example, the two recent attempts at deep-brain stimulation in the entorhinal cortex have produced opposite results – one has improved memory[13], whereas the other made it worse[14]. My belief is that these types of treatment will first require a solid understanding of the basic neural dynamics and how they relate to specific memory functions.

Answering these questions in the lab has been challenging for two main reasons: 1) Anatomy of the hippocampal formation is incredibly complex and distributed and 2) Memory-related behaviors are exceptionally hard to study in traditional model organisms. Food caching in chickadees might provide a solution to both problems. Chickadees cache astonishing numbers of food items – up to 5000 per day[15] – at scattered, hidden locations. In a brief caching episode, they form an accurate "episodic-like" memory and can retrieve that cache up to a month later[16,17]. With no training, they readily perform this behavior in the lab[18,19]. Individual instances of memory storage and recall occur at discrete and experimentally well-defined moments in time, providing an excellent opportunity to study the underlying time-aligned neural activity. To retrieve caches, chickadees require the hippocampal formation[20,21], which is embryologically homologous to its mammalian counterpart and shares similar neural circuitry[22–24]. Yet, the circuit in birds has simpler organization than in mammals and is particularly amenable to neural recordings and manipulations.

In my essay, I will first unwrap this information to demonstrate the anatomical and ethological advantages of the chickadee food-caching behavior. I will also describe our success in bringing this behavior into the lab and making it compatible with the full array of 21st-century neuroscience techniques. Later in the essay, I will describe some dream experiments that we should be able to perform with this new model system. This is truly a novel paradigm in neuroscience, and one that I hope is a good fit for the New Innovator Award program.

Throughout the essay, I use "chickadee" to refer to two related species from the chickadee family: the black-capped chickadee (*Poecile atricapillus*), which has decades of history in the behavioral literature[18–20,25], and the tufted titmouse (*Baeolophus bicolor*), which is larger and therefore more tolerant of heavier recording devices[26]. In my lab, we use these species interchangeably. Food caching in not a sexually dimorphic behavior[27], and we use both male and female birds. We plan to analyze every result for possible differences between sexes and to report results disaggregated by sex.

The hippocampal system is highly conserved across vertebrates

A major reason to study food cache memory is that it relies on a circuit homologous to the mammalian hippocampal system. Here, I use the classical definition of "homology" to mean that avian and mammalian hippocampi have a common embryological origin: across species, hippocampi develop from the same medial part of the telencephalon and express similar transcription factors[22,28] (Fig. 1). Besides this common origin, they also have similar connections to other brain regions[24], local circuit organization[29], and morphological cell types[30]. As I will describe in this section, my lab has even found striking similarities between neural activity in the mammalian and avian hippocampi. These observations give us confidence that our discoveries won't become an isolated set of knowledge about a specialized organism. Rather, our findings should generalize to hippocampal systems in other organisms that use memory, including humans.

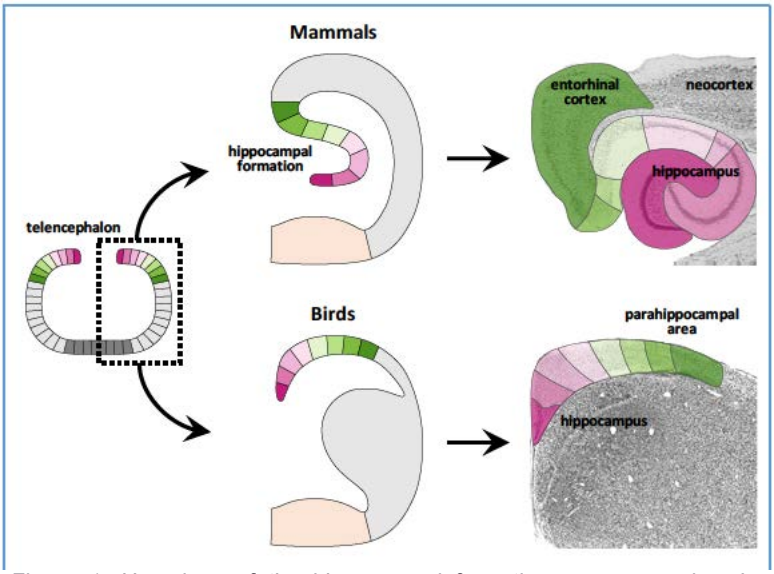

Figure 1. Homology of the hippocampal formation across species. In mammals and birds, the telencephalon forms by evagination of the neural tube during embryological development (left). Hippocampal formation, which includes the entorhinal cortex and the hippocampus, is the medial- most part of this region. In mammals, the hippocampal formation folds 3 times under the neocortex. In birds it instead develops as an unfolded structure on the brain surface. Similar sets of transcription factors are expressed along the transverse axis (green -> magenta).

The avian hippocampal system has attracted little attention from modern neuroscience, possibly because early recording attempts in birds have failed to find basic activity patterns that are usually studied in mammals. The most famous of these are place cells - hippocampal neurons whose activity is strongly localized in physical space[5]. Recordings in foraging pigeons instead found only weak and unstable spatial neural activity[31,32]. The second prominent pattern of hippocampal activity occurs during sharp-wave ripples - near-synchronous activations of neurons when an animal is immobile. These patterns have been linked to memory because they "replay" recent experiences and affect memory consolidation [5]. Yet, recordings in the bird hippocampus have also failed to detect sharp-wave ripples[36]. Based on these data, various authors have begun to suspect that mammals use fundamentally different neural mechanisms for memory than other vertebrates[37,38].

In starting my research program, I decided to reconsider these negative results for several reasons: 1) Unlike previous work, we focus on birds that naturally remember many cache sites, and for whom physical location is therefore an exceptionally salient variable. Firing patterns in the hippocampus vary across species

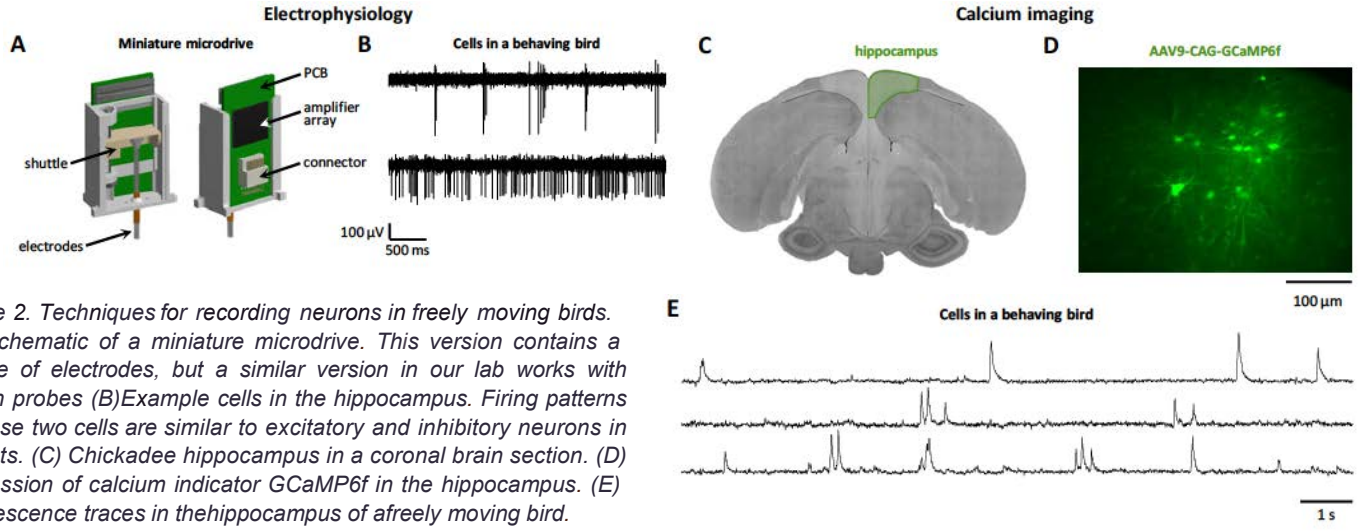

Figure 2. Techniques for recording neurons in freely moving birds. (A) Schematic of a miniature microdrive. This version contains a bundle of electrodes, but a similar version in our lab works with silicon probes (B)Example cells in the hippocampus. Firing patterns of these two cells are similar to excitatory and inhibitory neurons in rodents. (C) Chickadee hippocampus in a coronal brain section. (D) Expression of calcium indicator GCaMP6f in the hippocampus. (E) Fluorescence traces in thehippocampus of afreely moving bird.

and can depend on the ethological demands of specific animals[39,40]. 2) Using recent advances in miniaturized techniques, we can finally record simultaneously from large populations of neurons in birds - a feat necessary for detecting synchronous events like sharp wave ripples. 3) Finally, we now have a much better understanding of avian anatomy. Based on this knowledge, we specifically targeted the anterior hippocampus, which embryologically corresponds to the rodent dorsal hippocampus[41] - i.e., the region with the most prominent place cells[42,43].

Over the past couple of years, my lab has successfully developed techniques needed for this purpose (Fig. 2). The challenge we have overcome is allowing very small birds exceptionally unconstrained movement required for food caching. We have used miniaturized microdrives to record with electrode bundles and more recently with silicon probes. We have also optimized viral techniques to express the calcium indicator GCaMP6f in hippocampal cells and have recorded activity using head-mounted microscopes. This technique in particular takes advantage of bird anatomy: the avian hippocampus develops on the dorsal surface of the brain (Fig. 1) and can therefore be imaged without the customary removal of the overlying tissue[44]. In our first set of preliminary experiments, we simply allowed birds to search an environment for pieces of food, mimicking the standard "random foraging" protocol used in rodents.

Incredibly, our recordings indeed revealed place cells nearly indistinguishable from those found in rodents (Fig. 3). These cells were prevalent, but found almost exclusively in the anterior hippocampus - much like they are found almost exclusively in the dorsal hippocampus of rodents[42,43]. During periods of immobility, we also observed synchronous population events (Fig.4). In electrophysiological recordings, such syn-chronous events were accompanied by local field potential signatures nearly identical to the mammalian sharp-wave ripples[37]. Remarkably, these are the first ever recordings of place cells and sharp-wave ripples in the hippocampus on any non-mammal.

The implication here is that hippocampal mechanisms might be much more conserved across species than previously thought. This finding is important in its own right, and we plan to explore it further. For example, we will record from the part of the bird hippocampus that should be homologous to the entorhinal cortex (colored green in Fig. 1). The hypothesis is that this region will contain grid cells and other activity characteristic of the mammalian entorhinal cortex[6,45]. Yet, our findings are even more important for the goal of understanding how neural activity relates to memory functions. Nearly identical firing patterns across species will allow relating our findings to the vast knowledge about the mammalian hippocampus and will enable us to generalize our discoveries.

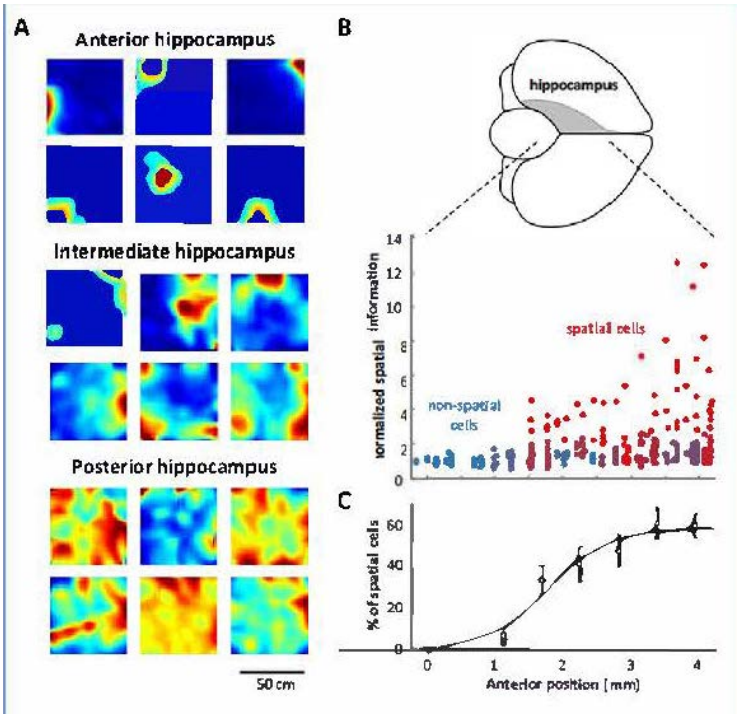

Figure 3. place cells in food-caching birds. (A) examples of cells from different locations in the hippocampus. each plot is a map of firing rate as a function of location. Color is from 0 Hz (blue) to maximum for each cell (red). (B) All hippocampal cells recorded along the anterior-posterior axis. Normalized spatial information in the mutual information between spiking and the bird's position, normalized by the mean value of this statistic in shuffled data. (C) Fraction of cells that pass the p < 0.01 threshold for being considered place cells. Place cells are almost exclusively found in the anterior hippocampus.

Taking advantage of a simplified circuit organization

In addition to discussing the similarities of mammalian and avian brains, I will mention a significant difference that has its advantages for our experiments. A major challenge to the study of the hippocampus is its extreme complexity and distributed anatomy. In rats, the hippocampal formation connects (primarily via the entorhinal cortex) to about 28 cortical regions alone[46]. These are often scattered, poorly demarcated regions. Attempting to understand such a circuit mechanistically is a daunting task. In birds, regions homologous to the cortex (shown in gray in Fig. 1) are instead organized into distinct clusters of cells called nuclei[23,47]. In addition to the same embryological and genetic origin, these nuclei have many properties similar to the cortical regions of mammals. For example, they receive sensory thalamic inputs and produce nearly identical sensory responses[47,48]. Yet, thanks to the well-separated and well-defined anatomy, nuclei are particularly amenable to

targeted recordings and manipulations.

In preparation for a detailed circuit analysis, my lab has begun tracing the inputs and outputs of the hippocampal formation in chickadees. We did this using both retrograde and anterograde tracers injected into various parts of the hippocampal formation (including the region that likely corresponds to the entorhinal cortex, colored green in Fig. 1). Consistent with data from other species[24],we found that only two cortical nuclei are connected to the hippocampal formation, abbreviated as HD and NL (Fig. 5). Both of these are known to be primarily visual regions, receiving direct and indirect inputs from the visual thalamus and the optic tectum[47,49,50]. This is in line with behavioral data showing that cache recovery relies exclusively on the memory of visual landmarks[18,19]. Essentially, what we have in these birds is the reduction of a large, distributed sensory system into a compact circuit that contains only two well-defined input nuclei. Needless to say, this circuit should be particularly tractable.

The outputs of the hippocampus are similarly confined to a small number of well-defined nuclei. I will describe these in more detail later, in the context of a proposed set of experiments.

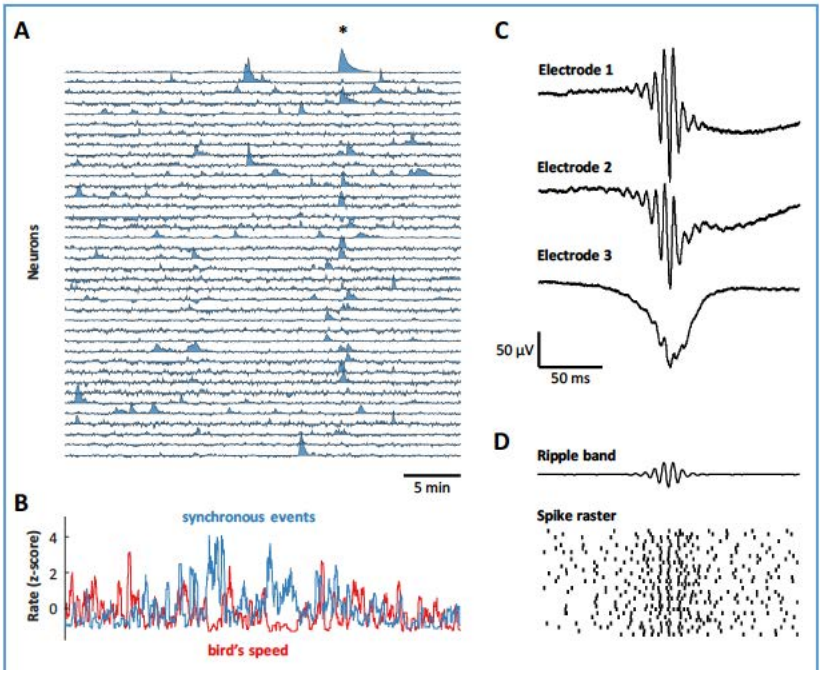

Figure 4. Sharp-wave ripples in the bird hippocampus. (A) Fluorescence traces from simultaneously imaged hippocampal neurons. Asterisk indicates a synchronous population event. (B) Average firing rate across all neurons and the bird's speed. Synchronous events occur primarily during immobility. (C) Local field potential (LFP) recordings across electrodes. Traces contain both a sharp-wave component (slow deviation) and a ripple component (fast oscillation). (D) Spikes of a single neuron aligned to increases in the ripple band of the LFP (150-250 Hz). The waveforms and the spike modulation are similar to sharp-wave ripples found in mammals.

Food caching is a neuroscience-compatible paradigm for studying episodic memory

Putting anatomy aside for now, I will discuss why behavior itself is a good reason to focus on food caching. The main motivation is that most animal behaviors in the lab fail to recreate basic features of human episodic memory. Humans are capable of storing multiple memories in a brief period of time. They often require a single exposure to form a new memory and can recall such memories flexibly, "on demand." In contrast, animal behaviors in the lab usually require extensive training, have rigid output (e.g. one of a small number of choices), or require low memory capacity. Training animals on increasingly complex cognitive tasks is very challenging, if not impossible. This is especially true if animals are asked to remember multiple unique items or events. Because memory is a covert process, it can also be hard for experimenters to know what exactly a subject remembers at a given moment in time. So even if an animal successfully forms multiple memories, it can be difficult to obtain a clear behavioral readout of these memories during an experiment.

Food caching is a remarkable solution to these problems. It is an innately complex behavior that requires minimal training. Even in the lab, its capacity can rival some aspects of human memory. It contains distinct moments of memory formation (caching) and recall (cache retrieval). These well-defined time points provide an excellent opportunity to study the underlying neural processes. Finally, cache memories are formed in a truly

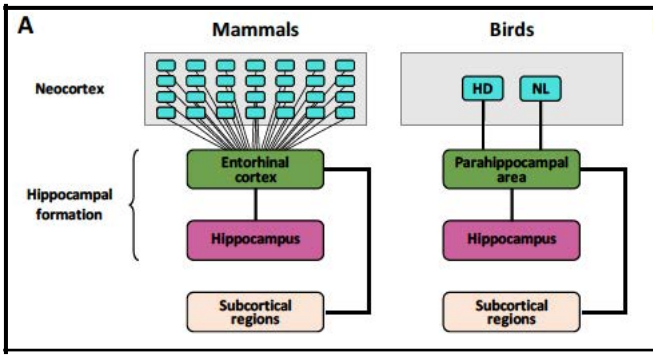

Figure 5. Extrinsic connections of the avian hippocampus. (A) *Basic circuit diagram, using the same colors as Fig. 1. In mammals, about 28 cortical regions are connected to the hippocampus. In birds, there are only two well-defined nuclei. Both nuclei are strongly linked to the visual system. (B) One of the inputs, labeled by retrograde tracer injections into the hippocampal formation (cholera toxin subunit B). HD: hyperpallium densocellulare; NL: nidopallium laterale. Scale bar:100 µm.*

"episodic-like" fashion - i.e., during a single event, without requiring any rehearsal or repetition. In psychology, food caching has been considered a textbook model of episodic-like or "what/where/when" memory because it pertains to a single-shot event (caching of a food item) and because birds remember the location, content, and even relative time of each cache[25, 51,52].

The experimental challenge is that chickadee food caching has traditionally been studied in large, naturalistic environments that are not easily compatible with neural recordings and manipulations[18-20]. Our goal is to bring this behavior into the lab and to make it compatible with the full power of 21st century neuroscience. We plan to chip away at this challenge from two ends. First, we will develop behavioral arenas that are neuroscience-friendly, but still allow birds to express their natural food-caching behaviors. At the same time, we will continue developing and optimizing methods for recording and manipulating neural activity in increasingly unconstrained settings. The goal is to reach a perfect compromise between natural behavior and advanced lab techniques. Thanks to the recent explosion of electronic and optical technologies, this goal has become realistic for food-caching birds.

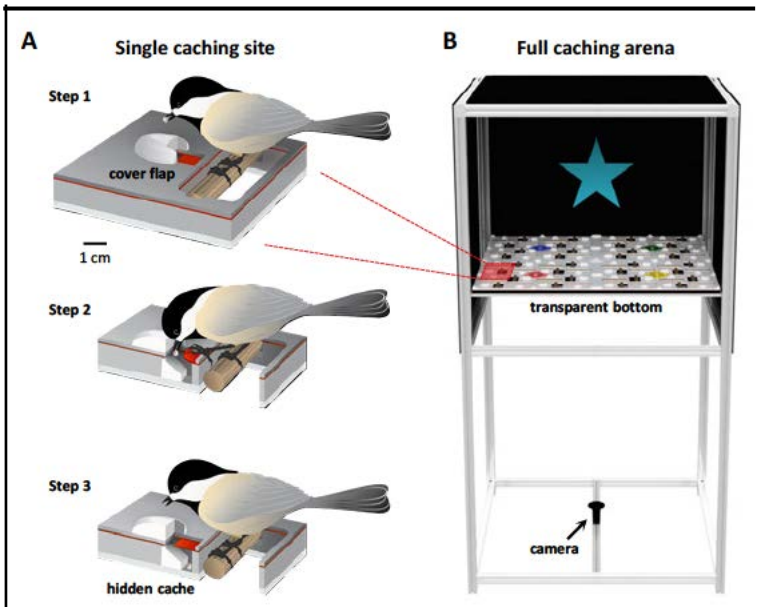

Figure 6. Automated lab arena for food-caching experiments. (A) Schematic of a single caching site. Chickadee places a seed under a flap. This behavior occurs without training. Once the flap snaps closed, the seed is invisible from the outside. (B) Full 60x60 cm arena containing 64 cache sites. Contents of all sites are seen by a bottom camera and automatically tracked by real-time video analysis software.

Fortunately, we have discovered in our early attempts that chickadees are fairly indiscriminant about caching food into artificial lab objects. We took advantage of this robust behavior to design an arena that contains a large number of ergonomic cache sites within a reasonably confined space (Fig. 6). Two features are critical about this design. First, the contents of these sites are invisible, requiring birds to investigate them by opening a cover flap. This feature provides a discrete and unambiguous readout of the bird's behavior. The second feature is that the bottom of the arena is transparent, allowing a single camera to track the contents of the cache sites and to detect when the bird is investigating them. By detecting food caches with real-time video processing, we have been able to make this arena compatible with closed-loop manipulations. I.e., motors, actuators, lights, digital displays, and potentially even neural stimulation or inactivation can be triggered by food caches and retrievals.

Taking advantage of these features, we demonstrated that chickadees indeed use memory to find caches in the lab. We showed this by designing a pilot behavioral paradigm in which birds cache a small number of seeds, receive a timeout period, and are then asked to find food (Fig. 7). Birds find their caches 2-5 times faster than expected from a random reshuffling of trajectories between behavioral trials (this analysis compensates for bird-specific spatial biases). It is known that this kind of performance requires memory of visual landmarks and not some proximal sense like olfaction: birds do not find decoy food items faster than chance and make predictable errors when visual landmarks are distorted[16,19]. Crucially for our research program, we used pharmacological inactivation to demonstrate that cache retrieval depends on the hippocampus (Fig. 7).

Our behavioral arena achieves a nontrivial technical feat. It allows birds to express a highly complex, memory-guided natural behavior with minimal training. At the same time, this arena has the efficiency and simplicity of operant training chambers that are the workforce of modern behavioral neuroscience: it is fully automated, compatible with many recording techniques, and can even be used for high-throughput experiments on multiple animals in parallel. These features set the stage for neuroscience inquiry into fundamentally new types of cognitive behaviors.

Understanding the neural signatures of episodic memory

In previous sections, I outlined our successes along two directions: bringing chickadee behavior into the lab and understanding the basics of avian hippocampal physiology. These efforts finally allow us to pursue the dream experiment: recording hippocampal activity when an animal is actively forming and recalling memories.

Our key question is very simple: what happens to hippocampal activity when a new memory is formed? We understand a lot about place cells and other cell types in the hippocampal formation. But these cells are almost always recorded in static conditions, once an animal has learned an environment and has been extensively trained. The critical goal is to link hippocampal activity to dynamic memory. Do place cell firing fields change if a certain location has become associated with a specific memory? If they do, how do these changes represent the content of the memory?

We plan to address these questions by recording hippocampal cells, allowing birds to cache seeds at multiple sites, and observing how representations of location change on subsequent visits to these sites. This is fundamentally an exploratory goal; anything we observe will be highly novel. But there are a couple of possibilities we can reasonably expect from existing models of the hippocampus:

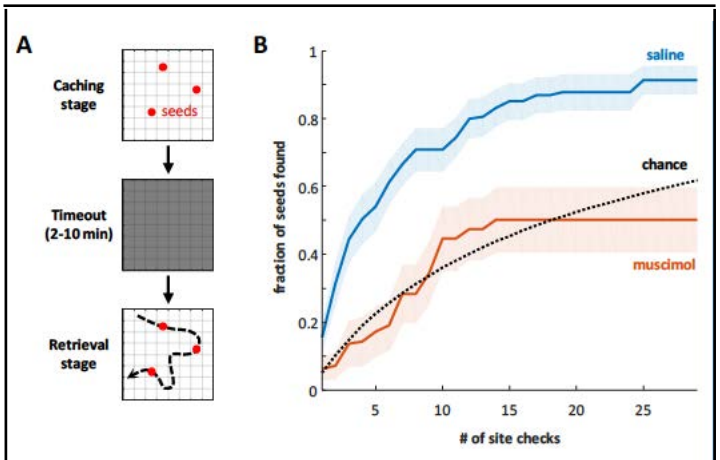

Figure 6. Chickadees use hippocampus-dependent memory to find caches in the lab. (A) Schematic of a behavioral trial. A bird takes seeds from motorized feeders, eating some and caching others. Once 1 – 3 seeds are cached, birds receive a timeout period (lights turned off). During the retrieval stage, feeders are closed, and previously made caches are the only source of food. Investigations of individual sites are automatically recorded. (B) Birds find seeds faster than expected by chance. Mean ± s.e.m. across all trials for one bird are shown. Chance is calculated by randomly shuffling bird's trajectories across trials, a procedure that preserves possible spatial biases. Muscimol was infused into the hippocampus on alternate days; on these days behavioral performance was at chance level.

Hypothesis 1: In one class of hypotheses, hippocampal firing undergoes immediate and persistent changes whenever a new memory is formed. For example, imagine a "sunflower seed-preferring" neuron. This neuron will become active at any locations where seeds are cached and will remain active there on all subsequent visits by the bird. There might also be "pure" place cells that are active at preferred locations regardless of the cache content. Finally, as is common in the hippocampal system[10,53], there might be "conjunctive" cells that have both place and food preference. These cells will appear to be place cells in our recordings, but will change their firing rates in a consistent way whenever seeds are cached or retrieved.

This model is inspired by a body of data showing that hippocampal neurons not only represent location, but also have activity correlated to a variety of sensory and internal variables[7-10]. It is also consistent with work showing that hippocampal cells have mechanisms for exceptionally fast plasticity and can exhibit sustained changes to their firing rates after a single behavioral trial[54,55]. Finally, this model is particularly inviting due to its mechanistic implications. During cache retrieval, another brain region can "query" remembered cache locations by encoding the representation of a sunflower seed at the inputs to the hippocampus. Via pattern completion[56], hippocampal activity would then recall one of the seed-containing locations.

Hypothesis 2: In an alternative class of hypotheses, cells specific to a given memory are context-dependent and only get activated when the bird is actively storing or recalling that memory. If these "memory" cells can affect place cells, we might still observe changes to place cell firing. For example, some models suggest that place fields will change firing rates or exhibit a bias in the direction of the recalled cache site[57,58]. However, these changes to place cell firing will be transient; for the rest of the time, the place map will be relatively stable. In the behavioral paradigm we developed, birds switch between periods of caching, foraging, and retrieving caches within a brief behavioral session compatible with neural recording (about 1 hour). We are therefore well positioned to detect transient changes of hippocampal activity, if such exist.

Once again, these are broad classes of hypotheses that contain many variations, only some of which have been entertained in the literature. Regardless of what we observe, we have a real chance of recording an interpretable hippocampal signature of episodic memory.

Understanding how memory recall influences behavioral output

Hippocampal firing patterns are only meaningful insofar as other brain regions can interpret them when memories are recalled. Connections to other brain regions are the only way the hippocampus can actually influence ongoing behavior. The critical goal is to understand what information is conveyed by hippocampal outputs, to which targets, and at what moments in time. Systems neuroscience is undergoing a revolution of pathway-specific analyses in the brain. In many cases, identifying specific outputs has revealed a clearer picture of a brain region with otherwise complex and heterogeneous activity[59,60]. Yet, incredibly, very little is

known about the function of specific hippocampal outputs. As I described above, this is likely due to the extreme complexity and distributed anatomy of this circuit. We can bring the much-needed clarity to this problem by using food-caching birds, in which hippocampal outputs are confined to a small number of well-defined nuclei and are needed for a well-defined behavior.

Thankfully, advances in pathway-specific techniques are now applicable to birds. For electrophysiology, we can identify output neurons using antidromic stimulation of hippocampal axons that target specific regions. Antidromic stimulation has been used in a number of systems, but has been especially efficient in the avian brain, where densely clustered and well-separated nuclei are ideal for targeted electrical stimulation[59,61]. For imaging, we can express calcium indicators in specific hippocampal output neurons by injecting retrogradely transportable viruses into target regions. Both techniques have now worked successfully in food-caching birds in my lab.

In our anatomical analysis, two outputs of the hippocampal formation are particularly dense and target conspicuous candidates for influencing caching-related behaviors. One of these is the striatum, which has been implicated in a variety of motor behaviors, including flying and hopping in birds[62]. This output is equivalent to the prominent projection of the entorhinal cortex to the striatum in mammals[63]. A second output is to the lateral hypothalamus. As in mammals, this region is involved in feeding behaviors closely associated with food caching[64,65]. There is a handful of additional outputs, but our initial work will focus on these two.

Our first goal will be to record hippocampal neurons that project to the striatum or the lateral hypothalamus immediately before and during cache retrieval – i.e., moments in time when the bird is actively using the hippocampus to recall a memory. A key feature of episodic memory is that a single memory binds diverse channels of information (e.g. spatial and non-spatial) about a given event. A critical question is whether distinct channels of information are at least partially unmixed in the outputs of the hippocampal formation. There are two distinct hypotheses:

Hypothesis 1: One possibility is that different types of information are conveyed to distinct hippocampal targets. For example, location-sensitive neurons might preferentially project to the striatum and contribute to the production of navigation-related movements. Meanwhile, food-specific neurons (like the “sunflower seed-preferring” neuron I described above) might preferentially target the lateral hypothalamus. Once the animal is at a given location, these neurons could contribute to the choice of an appropriate feeding behavior.

Hypothesis 2: Alternatively, the hippocampus might convey identical, general code to the striatum and the hypothalamus. In turn, targets receiving mixed location/content signals would extract information necessary to specific, local computations. Sending information to regions that are bound to ignore it may seem wasteful. But having general-purpose neurons in the hippocampus might make the network more flexible in learning new information and simplify learning rules that are engaged during memory formation. Which of these general design principles (Hypothesis 1 or 2) is followed by the hippocampus is an open question. It is a fundamental question for our understanding of how memory recall influences behavior.

In summary: a new paradigm for addressing challenging questions in neuroscience

From the mechanisms of action potentials to the basic principles of vision, neuroscience has often benefited from model organisms that have unique specializations. In most cases, these have been sensory specialists (as in the case of whisking rodents[66]) or motor specialists (as in the case of singing birds[59,67]). The chickadee is unique for being a *cognitive specialist*. The hope is that it will allow us unprecedented insights into episodic memory – a brain function for which current model systems are in many ways insufficient. The study of this system will allow analyses of neural activity that are not usually attempted, either because of circuit complexity or inadequate animal behavior in the lab. At the same time, comparisons to existing models will also allow us to disengage from possible idiosyncrasies of individual species and to discover truly fundamental principles of hippocampal function. Cross-species analysis has already been exceptionally fruitful in the hippocampus field[39,68].

The problem of episodic memory is particularly relevant today. Our understanding of cognition has dramatically advanced in recent years. We can now build biologically inspired machines that rival or even surpass our own brains in several domains[69]. Yes, machines often require weeks of training, whereas humans and animals can form memories rapidly, often from a single exposure. This discrepancy is telling: there is a fundamental gap in our knowledge of not only how episodic memory works, but even of how it *might* work. Indeed, some other fields of neuroscience benefit from unifying conceptual models (e.g. reinforcement learning as a model of motor learning[70]). In contrast, there is a striking lack of similar algorithmic models of rapid memory. Especially because episodic memory is often the first victim of devastating memory disorders, there is a dire need for basic biological observations of this process. The chickadee hippocampus might help fulfill this

need.

In conclusion, I will mention the ultimate goal that our new system may allow us to achieve. A dream of systems neuroscience is to create what has been called “gap-less” understanding of neural circuits. This means a complete understanding of the computations performed by the entire circuit – from sensory inputs to motor outputs. To date, this type of a description has only been possible for very simple circuits, almost exclusively in invertebrates[71]. I believe that by using the food-caching system, we have a shot at a gap-less circuit that includes memory and sophisticated cognition. The chickadee hippocampus is only three synapses away from the sensory (visual) periphery with well-defined, compact circuitry in between. It is similarly close to the motor outputs that drive feeding behaviors. We are, of course, far from the ultimate goal. But our experiments over the next five years will set the stage for this level of understanding in the future. Because the circuit is fundamentally the same as in mammals, lessons we learn will generalize to other fields – all the way to the human hippocampus.

Finally, establishing chickadee food-caching as a model system might create an entire subfield of neuroscience. Its reach could extend to basic and clinically-relevant questions beyond those that my lab intends to address in the next five years. For example, food caching has become a psychological model for social cognition and even the theory of mind, because birds show sophisticated ways of concealing and stealing caches from each other[72]. The chickadee hippocampus is also a site of intense adult neurogenesis[73] (a process that has actually been first discovered in birds), providing a model of how this process might relate to memory. The list does not end here. This is a daring direction, but one that is already proving to be fruitful. A New Innovator Award would be decisive for enabling this pursuit to go on.

INNOVATIVENESS

The proposed project is highly innovative in several respects. It introduces a model organism that has never been studied with modern neuroscience tools. It proposes to use some of the most cutting-edge technologies in high-density electrophysiology, functional calcium imaging, and pathway-specific circuit dissection. Unlike other fields where these techniques are becoming available off-the-shelf, the proposed project will have to push their limits. To study food caching, we need technologies to work in particularly small birds, during unconstrained navigation, and in wild-type subjects. Needless to say, there are some inherent uncertainties in getting this to work.

Our biggest risk from the onset was that food caching would not be compatible with laboratory environments, or that birds would use strategies alternate to hippocampus-dependent memory. Fortunately, this risk is now behind us: we discovered that the behavior is incredibly robust in artificial environments, and that hippocampal activity is necessary to retrieve food caches. Another risk was that food-caching birds would not tolerate recording devices. This risk is also largely gone, and we now routinely record hippocampal populations comparable in size to those typically studied in rodents. We have identified serotypes of recombinant viruses that work well in food-caching birds and have successfully expressed a variety of genes in their brains, including GFP, GCaMP6f and Channelrhodopsin-2. This preliminary work sets the stage for a wide variety of critical experiments, without leaving us methods-limited.

The most exciting uncertainty of this project is conceptual: we will need to remain highly innovative in the future interpretation of any data that we obtain. Thankfully, basic features of avian hippocampal activity in our preliminary experiments are starting to look familiar: we are observing activity analogous to the well-described mammalian phenomena like place cells and sharp-wave ripples. These findings give us a conceptual framework for generalizing our results and relating them to the existing literature in other species, including humans.

INVESTIGATOR QUALIFICATIONS

My previous research is a unique combination of extensive work on both small songbirds[4-10] and on the mammalian hippocampal formation[1-3]. These areas of expertise are uniquely synergistic for establishing food-caching birds as a new model in systems neuroscience. My proposal requires expanding both the tools for experiments on small birds and the framework for thinking about the novel data. Thanks to my prior experience, I am ready to tackle both the technical and the conceptual challenges. (Note: bibliographic citation numbers in this section refer to my Biosketch).

Technical strengths. In my graduate work with [redacted], I studied the vocal babbling of juvenile zebra finches. Neural recordings of fragile young birds in the babbling stage proved to be exceptionally challenging – much more so than the previous work on older birds. A decade ago, we also didn’t have the luxury of tools that are now available for such applications, like miniature multiplexing amplifiers or micron-precision 3D printing. I

successfully developed several novel technologies for use in these animals, including electrophysiology and pharmacology[4,6], surgical methods[5], Peltier cooling[10], in-brain temperature recordings[9], and respiratory measurements[8]. These tools allowed me to functionally dissect vocal pathways in birds at the very first stage of motor learning[4-6] and to record from particularly small and deep structures in the bird brain[7]. The "niche" engineering skills I acquired in the process have now been applied in numerous cases of preparing my lab to work on chickadees.

I continued technically intensive work in my postdoctoral fellowship with [redacted]. My first project required developing a virtual-reality system for rats. Previous systems for rodents constrained animals in one dimension (usually by head fixation) and failed to engage 2D firing patterns in the brain, like those of grid cells. I overcame the challenges imposed by free rotation and developed a system in which rats could turn and walk in any direction. This required designing a novel visual projection scheme, specialized hardware, new software, and a custom miniaturized recording system. I succeeded in making all these components work and published the first-ever 2D neural patterns recorded in virtual reality in *Neuron*[2] in 2014. Again, my experience building entirely new experimental systems from scratch has been invaluable for designing food-caching experiments.

Conceptual strengths. In several cases, my experiments and analyses have challenged existing beliefs in the field and led to reinterpretations of existing data. Prior to my work on songbirds, it had been assumed for more than 30 years that a vocal region called HVC drives singing at all stages of development. Upon developing the necessary tools for young juvenile birds, I found inconsistencies with this dogma. Instead, I carefully recorded and manipulated the circuit to show that early in development, a different region called LMAN drives the babbling behavior. This was a paradigm-shifting study (published in *Science*[6] in 2008) that has changed the way the field of song learning operates to this day.

In my postdoctoral work, I questioned the long-standing idea that highly spatial cells in the hippocampal formation (like place cells and grid cells) are uniquely specialized to represent physical location. I designed a new behavioral paradigm in which rats navigated along an entirely non-spatial auditory dimension, using a joystick to change sounds in their environment. I found that the activity of all spatial cell types, including place cells and even grid cells, represented sound frequency in this task. This finding was again highly surprising to the field and was published in *Nature*[1] in 2017.

SUITABILITY FOR THE NEW INNOVATOR AWARD PROGRAM

This proposal is an excellent fit for the goals of the New Innovator Award program. Rather than continuing to expand my well-established postdoctoral work, I chose to build my lab around a new set of experimental paradigms and a novel model organism. Chickadee food caching is a classic model in behavioral biology, but has not been tackled by modern neuroscience. Everything we do therefore requires innovation: design of behavioral setups, recording technologies, data analysis, and conceptual frameworks for interpreting the data. This type of work may be considered risky by the more traditional funding mechanisms. But it is also inherently a high-reward pursuit. We aim to perform the first-ever detailed dissection of a neural circuit in the act of forming and recalling episodic memories. At stake here is the possibility of bridging hippocampal activity with the critical medical role of this brain region in memory.

STATEMENT OF RESEARCH EFFORT COMMITMENT

If chosen to receive an award, I will commit a minimum of three person-months (25%) of my research effort to the project supported by the New Innovator Award.

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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

1) Description of procedures

The proposed experiments will use birds from the food-caching *Paridae* family (chickadees and titmice) to study the hippocampal mechanisms of memory formation and recall.

Two species used will be black-capped chickadees (*Poecile atricapillus*) and tufted titmice (*Baeolophus bicolor*). Birds will be wild-caught according to federal and state scientific collection licenses issued to Dr. Aronov by the Fish and Wildlife Service and the New York State Department of Environmental Conservation. We will use both males and females and perform experiments on adult birds (at least 3 months post hatch). We estimate using about 50 birds of each species per year for anatomical tracing, calcium imaging, electrophysiology, and behavioral experiments.

Birds will be housed in a state-of-the-art aviary at Columbia University's Jerome L. Greene Science Center and monitored daily by veterinarians. Surgical procedures will performed in a modified rodent stereotaxic device. Standard sterile techniques will be followed; this includes wearing sterile masks, gowns, and gloves, autoclaving all instruments, and sterilizing all implants with CIDEX (surgical-grade gluteraldehyde). Prior to all surgeries, birds will be intramuscularly injected with buprenorphine for analgesia. Birds will be anesthetized with 1-2% isoflurane in oxygen, and normal body temperature will be maintained using a homeothermic blanket. The level of anesthesia will be maintain by regularly monitoring breathing and the toe-pinch reflex. Small craniotomies will be made above the hippocampus or other regions of interest. For different experiments, either a replication-defective adeno-associated virus (AAV) or a neural tracer (Alexa-conjugated cholera toxin subunit B) will be injected, or a recording device will be attached to the skull using dental cement.

Behavioral and recording experiments involve a natural food-caching behavior and do not require any training or restraint. Birds will participate in experiments about 1 week after surgery, once they have fully recovered. They will be briefly food-restricted (3-4 hours) and then allowed to forage in an small arena where food is provided by motorized feeders. In some experiments, the arena will also contain sites for caching food. Depending on the exact experimental design, feeders may periodically close for up to 10 min, requiring birds to retrieve their caches.

2) Justification

The formation and recall of episodic memories are processes that happen in the brains of awake, behaving animals. We cannot use *in vitro* preparations because the fundamental goal of this research is to relate neural activity to behavior. Not enough is currently known about the biology of these processes to allow simulating them with sufficient detail in computer models.

We chose to use the food caching behavior of birds to study episodic-like, hippocampus-dependent memory because this behavior contains well-defined moments of memory storage and recall. Birds naturally perform many repetitions of this behavior in a single recording session, providing an unprecedented opportunity to study the underlying neural mechanisms. Such tractable episodic memory paradigms are currently very hard to achieve with other model organisms. For behavioral studies, we chose black-capped chickadees because their food caching has been studied in detail for the past 30 years, with a lot of basic information available in the literature. These birds are extremely common and adapt well to laboratory condition. For recordings, we chose tufted titmice because they are closely related to chickadees and exhibit similar behaviors, but are twice as heavy and can therefore tolerate larger recording devices with ease.

Finally, food-caching birds perform memory behaviors with neural circuits that are homologous to the mammalian hippocampus. Our discoveries will therefore likely generalize to other memory systems and be relevant for understanding human brain function in health and disease.

3) Minimization of Pain and Distress

Collection. Birds will be attracted with feeders and captured using standard mist nets designed for chickadee- and titmouse-sized birds that do not cause any harm to the animals. Nets will be continuously monitored, and birds will be removed immediately upon capture and transferred to cages for transport. For 4-6 weeks from arrival to the Jerome L. Greene Science Center, birds will be treated for any parasites by the veterinary staff and gradually habituated to laboratory food.

Peri- and post-surgical care. Prior to each surgery, birds will be intramuscularly injected with buprenorphine for analgesia. Birds will be anesthetized with isoflurane in oxygen, and the sufficient depth of surgical anesthesia will be maintained by testing for the toe-pinch reflex every 5 min. After surgery, birds will be monitored every hour for the next 12 hours and then twice a day for the next 5 days. Signs of possible pain or distress include lack of eating or drinking, puffed up feathers, and inhibited movement or perching. In case any

of these signs are observed, on-call veterinarian will be immediately contacted for further course of action, which may include additional doses of buprenorphine or recommended euthanasia. Following implant or injection surgeries, the site of the incision will additionally be closely monitored until full healing. In the case of any irritation or inflammation, triple antibiotic ointment will be applied twice a day. All of the post-operative monitoring will be done by the person who performed the surgery, including on weekends and holidays. Veterinary staff are also available on campus at all times.

Behavioral and recording experiments. Food caching is a natural behavior for chickadees and titmice and does not require stressful training or restraint. To motivate caching and feeding behaviors, birds will be food deprived for 3-4 hours prior to the experiment. This is a relatively brief amount of time that is substantially shorter than typical food deprivation periods experienced by these species in the wild. To ensure that birds tolerate this feeding schedule well, they will be weighed daily. Ad libitum days will be provided for chickadees if they lose more than 0.5 g of weight over the course of one day or more than 1.5 g since the start of the experiment. For the heavier titmice, these criteria are doubled (1.0 g and 3.0 g, respectively). Birds will be monitored daily by on-duty lab personnel for decreased activity, puffed up feathers, or any other abnormalities. In case of any signs of pain or distress, birds will be immediately provided with ad libitum food and will not participate in experiments until their condition fully recovers. If the condition doesn't recover in the course of one day, birds will be euthanized.

4) Euthanasia

Birds will be euthanized by transcardial perfusion with phosphate buffered saline, follow by 4% paraformaldehyde. Prior and during this procedure, they will be deeply anesthetized with ketamine/xylazine. The depth of anesthesia will be confirmed with the toe-pitch reflex. All procedures will performed by highly trained lab members. All methods are consistent with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Authentication of Key Biological and/or Chemical Resources

Biological resources used for the proposed project include two recombinant viruses: scAAV9-CBh-GFP and AAV9-CAG-GCaMP6f. Both are off-the-shelf products provided by commercial suppliers UNC Vector Core and Addgene, respectively. A retrograde tracer Alexa-conjugated cholera toxin subunit B provided by Invitrogen is also used. All of these products have been used extensively by the field and have been validated in our laboratory.