PI: **MCCLUSKEY, LYNNETTE Marie**	Title: Ace2 in the healthy and inflamed taste system	
Received: 08/05/2021	Opportunity: PA-20-195	Council: 01/2022
Competition ID: FORMS-F	FOA Title: NIH Exploratory/Developmental Research Grant Program (Parent R21 Clinical Trial Not Allowed)	
1R21DC019832-01A1	Dual:	Accession Number: 4608611
IPF: 676605	Organization: [redacted]	
Former Number: 1R21DC019832-01	Department: Neuroscience and Regen. Med.	
IRG/SRG: NIC	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
LYNNETTE MCCLUSKEY	[redacted]	PD/PI
Lin Gan	[redacted]	MPI
Daniel Linder	[redacted]	Co-Investigator
Camille King	[redacted]	Consultant
Michael King	[redacted]	Consultant
Xin-Yun Lu	[redacted]	Consultant

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
DC019832

b. Agency Routing Number

2. DATE SUBMITTED

Application Identifier
37562

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 9666686910000

Legal Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Sheree Middle Name: Last Name*: Wright Suffix:
Position/Title: [redacted]
Street1*:
Street2:
City*: [redacted]a
County:
State*:
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

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

7. TYPE OF APPLICANT*

M: Nonprofit with 501C3 IRS Status (Other than 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*
Ace2 in the healthy and inflamed taste system

12. PROPOSED PROJECT
Start Date* 04/01/2022
Ending Date* 03/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
[redacted]

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: LYNNETTE Middle Name: Marie Last Name*: MCCLUSKEY Suffix:
Position/Title: Professor
Organization Name*: [redacted]
Department: [redacted]
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email* [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

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

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

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

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Sheree Middle Name: Last Name*: Wright Suffix:
Position/Title*: Interim Executive Director
Organization Name*: [redacted].
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number [redacted] Email*: [redacted]

Signature of Authorized Representative*
Sheree Wright

Date Signed*
08/05/2021

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:2021.8_GanCoverLetter_ACE2.pdf

424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: [redacted]
Duns Number: [redacted]
Street1*: [redacted]SITY
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00197

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*	McCluskey_Abstract_2.pdf
8. Project Narrative*	McCluskey_Narrative_2.pdf
9. Bibliography & References Cited	McCluskey_Bibliography_FINAL.pdf
10.Facilities & Other Resources	McCluskey_Facilities_2.pdf
11.Equipment	McCluskey_Equipment_2.pdf

Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

PROJECT SUMMARY / ABSTRACT

Taste deficits are prevalent in people infected with SARS-CoV-2, the virus responsible for the global COVID-19 pandemic. The loss of taste sensation negatively affects nutrition and quality of life and in some patients this deficit is long-lasting. The biological basis for taste loss due to SARS-CoV-2 is largely unknown. Our preliminary results demonstrate that the ACE2 receptor and TMPRSS2 which together mediate SARS-CoV-2 host cell entry are expressed in taste buds indicating their potential for viral infection. The function of taste cell ACE2, also a member of the renin-angiotensin system that regulates fluid balance, is unknown. We have developed three novel genetic mouse strains to overcome the limitations of currently available mouse models. In aim 1 we map ACE2 reporter expression to determine which taste receptor cell populations and pathways are potential targets of SARS-CoV-2. In aim 2 we test how lingual epithelium-specific ACE2 contributes to taste receptor cell dynamics and neurophysiological taste responses under baseline and inflammatory conditions. We will also test how taste function is affected by human SARS-CoV-2 spike protein in a humanized ACE2 knock-in mouse. Our hypothesis predicts that taste buds are SARS-CoV-2 targets, that taste ACE2 contributes to taste function and is protective during inflammation, and that SARS-CoV-2 spike protein will exacerbate damage in taste buds and depress neural taste responses under inflammatory conditions. This R21 Exploratory / Developmental grant application addresses the urgent need for fundamental insights to mechanisms underlying taste dysregulation in people with COVID-19.

NARRATIVE

Taste loss is a common symptom of infection by SARS-CoV-2, the virus which causes COVID-19 by using the angiotensin converting enzyme (ACE)2 receptor to enter host cells. We will use novel mouse models to map ACE2-expressing cells in the taste system and determine the receptor's role in taste function under normal and inflammatory conditions. These studies will provide much-needed insight to mechanisms responsible for taste dysfunction due to SARS-CoV-2.

FACILITIES AND OTHER RESOURCES

Intellectual Environment. There are currently 24 primary faculty in the [redacted]. Faculty recruitment is a priority with 7 faculty hired since 2019 and searches ongoing. Neurologic disease is a major area of research emphasis at MCG, including over 60 neuroscience faculty. Neuroscientists are located in multiple clinical and basic science departments, research units, and institutes at the medical campus. The PIs' home department is largely focused on neuroscience research and targeted recruiting has strengthened the focus on neural degeneration and regeneration. Thus, there are excellent opportunities for interaction with other neuroscientists including groups studying sensory systems, spinal cord injury, and neuroimmunology. New core facilities, including Dr. Gan's Transgenic and Gene Editing Core Laboratory, used to generate novel mouse models for this application, are another benefit in the growing department. We also enjoy frequent interaction with external experts as part of the weekly [redacted] seminar series, an annual Neuroscience Retreat, and numerous other weekly seminars and grand rounds, now held in virtual formats. Research in immunology is based in the Cancer, Immunology, Inflammation & Tolerance (CIT) group within the Cancer Center, and Dr. McCluskey has established relationships with this group as the former director of the Graduate Program in Molecular Medicine which focuses on immunology. Dr. McCluskey now directs the Graduate Program in Neuroscience which provides frequent interaction with student and faculty neuroscientists. In sum, the PIs' research programs have excellent collaborative and intellectual support for both the neural and immune components of the proposed project.

McCluskey Laboratory. Dr. McCluskey's laboratory occupies ~1100 square feet in CA-3059. The laboratory is equipped with two neurophysiology and surgical stations, and image analysis system for fixed tissue and cell quantification, an image analysis system for measuring fluorescence in live cells and tissues, Bio-Tek plate reader, Leica cryostat, fume hood, -20ºC and -80ºC freezers, a water purification system, and molecular/biochemistry equipment. Benchtop and desk space is available for up to 10 personnel.

Gan Laboratory. Dr. Gan's main laboratory occupies approximate 2,000 sq. ft. in the adjacent [redacted] within the [redacted] The laboratory is fully equipped for tissue culture (Thermo Scientific biosafety cabinet/CO2 incubator), molecular biology and biochemistry (Thermo Scientific -80ºC freezer, Milli-Q IQ700 water purification system, NanoDrop One^{C} for DNA and RNA quantification, Eppendorf 5804 benchtop centrifuges, Eppendorf 5424 and 5424R microcentrifuges Bio-Rad QX200^{TM} Droplet Digital PCR, Bio-Rad CFX qPCR system, Bio-Rad C1000 thermal cyclers, Diagenode Bioruptor sonicator, Bio-Rad Gel Doc XR+, Bio-Rad semi-dry blotter, Eppendorf Vacufuge Plus, bacterial incubators and shakers), immunohistochemistry and in situ hybridization (Cryostar NX50 cryostat, Hybaid rotating/shaking ovens), embryo/tissue dissection stations (Nikon SMZ1500 and SMZ-U stereoscopes), bright light/DIC/pifluorescent imaging stations (Nikon TE2000U inverted microscope with DS-Ri2 camera and Zeiss Lumar V12 stereoscope with AxioCam camera), NEPAGENE NEPA21 in vitro & in vivo electroporator for cultured cells or explants.

Animals. The [redacted] maintains a centralized animal care program with administrative responsibility assigned to the Office of Laboratory Animal Services. Animal facilities for this project are located in the building adjacent to the PI's lab, accessible by a short hallway and service elevator. The facility and program have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) since May 16th, 1978. [redacted] is a Registered Research Facility with the United States Department of Agriculture (USDA No. 57-R-0002), and has an Office of Laboratory Animal Welfare OLAW Assurance Statement on file (D16-00197-01; valid until August 31, 2023). Importantly for the proposed studies on immune-gustatory interactions, the animal facilities are specific-pathogen-free (SPF). The routine animal husbandry and health monitoring services are provided by experienced vivarium veterinary staff consisting of several laboratory technicians and full-time veterinarians.

Offices: The PIs' adjacent offices occupy approximately 140 square feet each in [redacted] and [redacted], home to the [redacted] The PIs' offices are near their laboratories in a wing with five other neuroscientist faculty members. Additional Institute faculty offices and laboratories are located on the floors immediately above and below. Dr. McCluskey's office is equipped with a new Dell Optiplex and a Color LaserJet Pro printer. Dr. Gan's office is equipped with one MacBook Pro laptop computer and one

HP Laserjet 3525DN printer. The [redacted] provides staff support, copier, FAX, and phone services. Co-Investigators Dr. Bloomquist and Dr. Linder have offices are located nearby in the Dental College of Georgia and the Department of Biostatistics, respectively.

Computers:
The McCluskey Lab is equipped with 5 Dell Optiplexes, 3 Dell Latitude laptops, HP color printers and Dell laptops for each neurophysiology rig. Each computer has Photoshop, GraphPad Prism, Origin, EndNote and other software. One desktop computer is equipped to run Nikon's NIS Elements software for confocal image analysis. The majority of computers in the department were refreshed in 2020.

The Gan Lab is equipped with multiple common Apple and PC computers for all students and postdocs. The computers have software for word processing, spreadsheets, statistical analysis, graphics, and DNA analysis. The computers are connected to the internet and HP network laserjet M533DN printer. Each computer is installed with word processing, Photoshop, and other data analysis software. Two imaging microscopes are each equipped a dedicated PC computer, digital camera and softwares. The IT department at [redacted] provides technical assistance for electronic data management and hardware maintenance.

Other: The PI's home Department provides many high end, shared equipment that are relevant and available for this application, including Zeiss LSM700 confocal microscope system with software, Miltenyi Biotec UltraMicroscope II light sheet microscope for fast imaging of large 3D biological samples, two Nikon A1R-MP multiphoton microscopes with Mai Tai oscillators/multimaniuplator systems/patch clamp amplifiers, Keyence BZ-X810 fully-motorized all-in-one fluorescence microscope, and LI-COR Odyssey® CLx Imaging System for western blots. In addition, the Department provides free shared common equipment such as autoclave, glassware washing facility, high speed and ultra centrifuges (Beckmann and Sorvall).

Departmental Facilities:
Transgenic and Gene Editing Core Laboratory (Dr. Lin Gan, Director) is a full-service facility using state-of-the-art facilities and equipment for the creation of novel transgenic, knockout, knock-in, and conditional knockout mouse strains. The Core's facilities and equipment are located in CA3056, next to the PI's laboratory, and include two Nikon TE2000-S inverted microinjection microscope setups equipped with Eppendorf FemtoJet 4i electronic microinjectors/Nomarski Optics/Narishige micromanipulators for microinjections, Nikon surgical microscopes for egg and embryo isolation and transfer, Sutter P1000 micropipette puller for microinjection pipette preparation, biosafety cabinet/CO2 incubator/cell counter/electroporation system for ES cell culture and gene targeting, and C1000 PCR for mouse genotyping analysis. The Core's surgical and mouse housing facilities are located in Suite CA1117 on the 1st floor of CA building. The Core maintains all mice necessary for egg donors, egg recipients, and vasectomized males.

Biostatistics and Bioinformatics Core (Director, Yang Shi, PhD). The Biostatistics and Bioinformatics Core is a shared departmental resource for biostatistics and bioinformatics consulting and support ranging from project design to data analysis. It is a critical component of the research infrastructure in the Department of Neuroscience & Regenerative Medicine. Statistical analyses provided include statistical design of biomedical, translational and clinical studies, including sample size and power analysis; testing of assumptions of statistical tests; applying regression-based models to identify factors associated with disease outcomes; development of novel statistical methods and models; and bioinformatics analysis for Bioinformatics Analysis RNA-seq, microarray, ChIP-seq, DNA methylation, and whole genome sequencing.

Viral Vector Core (Director, Quansheng Du, PhD) The mission of the Viral Vector Core is to provide technological resources to [redacted] faculty members interested in the use of viral based vectors for basic research and preclinical applications. The core is supported by the DNRM Research Enrichment Funds. The vector core focuses on producing lentiviral, gammaretroviral and recombinant adeno-associated viral (rAAV) particles for transgene expression, gene silencing and gene editing in either dividing or non-dividing cells. The following viral vectors are available from the core: lentiviral vectors, adeno-associated viral vectors and gammaretroviral vectors.

Microscopy Facility (Director, Sergei Kirov, PhD). The imaging core is equipped with a Nikon A1R MP+ Multiphoton/Confocal Microscope with four high-sensitivity detectors for multiphoton imaging, seven visible

laser lines for confocal imaging, a MaiTai eHP DS ultrafast laser, full electrophysiology recording capabilities, fast imaging: 420 frames/sec maximum speed, high-speed piezo-drive focus motor, simultaneous photostimulation and imaging, and a best-in-class two-photon objective. Another Nikon A1R MP+ Multiphoton/Confocal Microscrope has four visible laser lines for confocal imaging and the same capabilities as A1R system above except for electrophysiology. The facility also includes a Nikon C1 Confocal with three visible laser lines and an imaging workstation with deconvolution software (NIS Elements)

Light-Sheet Microscopy Core. (Director: Alexis Stranahan, PhD) The [redacted] operates an Imaging Core Facility with a newly installed Lavision Ultramicroscope II light-sheet microscope (Miltenyi Biotec). The Ultramicroscope II has an infrared laser and a second tunable laser for acquisition of up to three excitation channels (488/560/633). This system is capable of imaging samples up to 8x8cm in aqueous or organic media in Zoom Body or Super Plan configuration. In the Super Plan configuration with the 12x objective, this system can acquire images with a maximal XY resolution of 0.217 microns/pixel. Resolution in the z-axis is determined by sample size, clearing efficacy, and laser configuration, but optical sections can be acquired at user-specified intervals for subsequent registration, segmentation, and high-throughput morphometric analysis. File size for tiled image acquisitions can reach 500GB per sample, and Augusta University provides unlimited cloud storage on an internal secure ftp site for data transfer and backup purposes. The departmental imaging facility also has two analysis workstations; one with Neurolucida 360 Studio for automated neuron tracing, dendritic spine and synapse detection, and a second with NeuroInfo software for registration onto the Allen Brain Atlas, automated segmentation, and cell detection (both from Microbrightfield).

Augusta University Medical College of Georgia Shared Core Facilities: All facilities are within the Medical Center complex.

Electron Microscopy and Histology Core Laboratory: The mission of the Electron Microscopy and Histology Core is to provide high quality services at an affordable cost to research investigators at the Augusta University and external research facilities. **The core** occupies ~3,000 sq. ft. and is located on the first floor of the Carl Sanders Research and Education Building (CB1113). The Core is well equipped and offers a variety of specimen preparation services for transmission and scanning electron microscopy, vibratome, paraffin, cryostat and JB-4 sectioning. The Core, which operates on a fee-for-service basis, offers routine H&E staining and various histological special stains, PASH, Masson Trichrome, Van Gieson and many others. The Core offers investigators a full line of immunohistochemical services including immuno-EM studies. It is a full-service facility, and augments research by providing expensive, specialized equipment and experienced technical staff to perform ultrastructural analyses. The Core staff performs all aspects of the ultrastructural procedures from specimen processing to imaging (for Electron Microscopy, TEM and SEM) and processing, sectioning and staining for light microscopy (paraffin, cryostat, and JB-4). The core is managed by Ms. Penny Roon (HT, ASCP). Additional staff members include Dr. Brendan Marshall, an expert in Electron Microscopy and Immunohistochemistry; Ms. Elizabeth Perry (MSA Certified), an expert in Electron Microscopy (TEM, SEM and IEM) and Immunohistochemistry; and Ms. Donna Kumiski, an ASCP Cerified Histotechnologist. Newly added services include sectioning of calcified bone specimens and EM-cryosectioning for EM-cryoimmunodetection.

The Genomics Core Laboratory is located on the [redacted] campus in the Interdisciplinary building (CA1041). It is a multifunctional molecular biology resource facility directed through the Center of Biotechnology and Genomic Medicine. Services are available to all campus investigators, students and incubator tenants, as well as off-campus users. Instruments available to users include: Life Technologies 3730XL genetic analysis instrument, Illumina Bead Station micro array instrument, Nano-Drop micro spectrophotometer, Fluidigm high throughput microfluidic-based genetic analysis instrument, Agilent Tapestation 2200 bioanalizer, Ion Torrent PGM next gen sequencer, Life Technologies 7900HT high throughput real time PCR instrument, RNA-seq, ChIP-seq, targeted re-sequencing, and de novo assembly, on the Illumina HiSeq and Roche 454 GS-FLX platforms. For automated fluorescence DNA sequencing the state-of-the-art ABI 3730 XL 96-capillary sequencer is available for ultra-high throughput projects and the ABI Big Dye Terminator 3.1 is the default instrument also available to users. The staff is qualified in assisting investigators in analysis and interpretation of their results.

Flow Cytometry Core: The AU Campus Flow Cytometry Core Facility (CFCCF located in CA2056) is equipped with flow cytometer equipment of 2 types 1) Cell Sorter - operated as a service to Investigators and 2) Cell Analyzer(s) - generally Investigator operated but can also be provided as a service as well. The Cell Sorter is

the Beckman Coulter MoFlo and allows for 7-color fluorescence, 4-way or multiwell plate cell sorting and has recently been upgraded to allow for sorting of well characterized BSL2 materials (ATCC, NIH, etc.) approved by the Biological Safety Office in addition to the BSL1 materials it has historically sorted. The Cell Analyzers, two bench-top BD (BD Biosciences) FACSCalibur instruments, are capable of 4-color fluorescent analysis. Computers workstations attached to the analyzers are available for data analysis, presentation and publication preparation when not in use for acquisition purposes; additionally, there are 2 standalone MAC's and one PC available in a separate analysis room as well. There are several softwares available for analysis purposes; BD CellQuest Pro, Treestar Flowjo, Verity Software House (VSH) ModFit in addition to MS Office to aid in presentation preparation. Core personnel are available to aid in experiment design and implementation as well as data preparation.

Cell Imaging Core: The mission of the cell imaging core is to offer investigators the use of state-of-the-art imaging equipment at a reasonable cost with expert training assistance if needed. The core, which is located in CB2201, offers confocal microscopy, multiphoton microscopy, digital light microscopy, computation, ratiometric analysis, image enhancement, 3D reconstruction, deconvolution and microinjection photo-uncaging and super resolution microscopy. The state-of-the art Carl Zeiss confocal microscopes offer imaging of live cells, thick sections and whole mouse studies. The Nikon Eclipse Ti super resolution scope equipped with STORM technology was recently installed. The specific instruments and their capabilities include: **(1)** Zeiss LSM510 META Axiovert 200M confocal microscope, LSM software; **(2)** Zeiss 780 Upright Confocal Microscope with GaAsp detectors, modules include - 3D, Deconvolution, HDR, Tiling, atmosphere (CO_2/O_2) and temperature control (for live cells); **(3)** Zeiss 780 Upright Confocal Microscope with the Definite Focus feature, modules include Physiology, FRET, FRAP, 3D, Tiling; **(4)** Zeiss 780 Upright Multiphoton Microscope, a 2-photon confocal instrument for visualizing thick samples. It features a fixed stage suitable for whole mouse studies. All but one lens are 'dipping lenses', (image while immersed in saline-bathing live samples). This system offers temperature control in 35-60 mm dishes. Its modules include: Physiology, FRAP, RICS. This application will primarily use the Zeiss 780 upright microscope with lasers: 405 nm, 458, 488, 514 nm, 543 nm, 633 nm. It is equipped with five lenses: 5x EC Plan-Neofluar(dry) - 0.16 NA, 10x EC Plan-Neufluar(dry) - 0.30 NA, 20x Plan-Apochromat(dry) - 0.8 NA, 40x Plan-Apo(oil) - 1.4 NA, 63x Plan-Apochromat(oil) - 1.4 NA. Owing to its two GaAsp detectors this instrument offers excellent resolution and outstanding sensitivity. Image analysis utilizes Zen 2012 imaging software. **(5)** Nikon Eclipse Ti research inverted microscope equipped for super resolution microscopy using STochastic Optical Reconstruction Microscopy (STORM) technology. The system is under the control of the universal NIS-Elements platform and features the SR Apochromat TIRF 100X 1.49 N.A. objective lens, which provides the highest quality point spread function for N-STORM super-resolution imaging. Expert microscopists assist investigators in the use of this equipment. They train investigators (or members of their lab) in the use of the instrumentation, or work directly with investigators to obtain images on a fee for service basis.

[redacted] ***Integrated Genomics Shared Resource:*** This resource offers both Next Generation Sequencing (NGS) and Microarray technologies and data analysis. For NGS analysis, the facility equipped with Illuminas High-Seq 2500, MiSeq , Next-Seq and a Life Technologies Ion Torrent. The HiSeq 2500 permits high-throughput sequencing output generating up to 200Gb data per run or 2 billion paired end reads per run. The cBot cluster generation station is also available for automated generation of clusters. For high through-put computational analysis the Resource is equipped an IlluminaCompute analysis and storage system with 2 Rack and 8 Blade servers with 36 AMD 16-core CPUs and 64GB RAM from Dell Corporate running Linux, dedicated of computational analysis. This cluster includes a 2-way SMP system acting as front-end cluster nodes and a file server, a large memory 2-way SMP system acting as a web front-end and a database server. Storage with approximately 60TB of raw storage capacity is shared out to the computational nodes. All file systems are built on top of a parity RAID scheme using RedHat XFS. The core personnel will prepare samples and libraries or these can be user generated. The core personnel are well-versed at running the following applications: Targeted Sequencing analysis, Gene Regulation Analysis, Sequencing-Based Transcriptome Analysis, SNP Discovery and Structural Variation Analysis, Cytogenetic Analysis, DNA-Protein Interaction Analysis (ChIP-Seq), Sequencing-Based Methylation Analysis, Small RNA Discovery and Analysis. The Facility also has access to a variety of software including the TopHat suite of analyses algorithms, ANNOVAR, BWA, GSNAP, GATK 2, Partek Genomics Suite and CLC Bio Genomics Workbench which can be accessed by individual users. In addition, the Bioinformatics core has 2 individuals assigned specifically to perform novel and downstream analysis of Next-Gen sequencing data. The Facility computers also contain Illumina software, Partek Genomics Suite, Ingenuity Pathways Analysis and a wide range of freeware analysis programs which can be accessed by individual users

including GATK 2.0 and the Tuxedo suite of programs for RNA-Seq data. In addition, the Bioinformatics core has 2 individuals assigned specifically to perform novel and downstream analysis of Next-Gen sequencing data. The GCC Integrated Genomics Facility also provides Affymetrix microarray technologies to faculty. The equipment and personnel are located within the GCC Cancer Center. The Resource aims to provide researchers with access to microarray technology and bioinformatics at an affordable cost, and to provide training to the research community through educational seminars for analysis of microarray data and programs designed to analyze large data sets. Equipment in this facility includes an Affymetrix GeneChip Scanner 3000 7G Plus, an Affymetrix Hybridization Oven 640, two Affymetrix Fluidics Station 450's, an Agilent Microarray Scanner, an Agilent Hybridization Oven, an Agilent 2100 Bioanalyzer, an Applied Biosystems GeneAmp 9700 Thermocycler, and a NanoDrop 1000 spectrophotometer. DNA sequencing and synthesis is also available. The available softwares for data analysis include; Affymetrix Genotyping Console, Affymetrix Expression Console, Affymetrix GCOS, GTYPE and CNAT, Clustall, CNAG, GO, Ingenuity Pathway Analysis, OGT CytoSure Visualization Software, Partek Genomics Suite, TreeView and Exon Easy. The facility personnel are well versed in the use of these various softwares for data analysis. The Pyrosequencer, and QPCT and associated software to be used in the epigenomics research projects is also located in this facility.

[redacted] offers collaborative research and services in the areas of bioinformatics. The staff has experience and expertise in bioinformatics software/database development, customized data analysis, and providing bioinformatics infrastructure and training. Services include, but are not limited to: development of bioinformatics software and databases, integrative analysis of multi-dimensional high-throughput data (e.g. gene expression, array [redacted] CHIP-chip, DNA methylation tiling arrays, SNPs, DNA sequencing data, metabolomics, proteomics etc.), identification of transcriptional regulatory elements, mapping of pathways and gene ontology, gene annotation, identification of alternative splicing forms, etc. The Resource has a seamless partnership with the Department or Biostatistics at MCG coordinating the effort of providing bioinformatics service/support to the local biomedical research community. The Resource staff are responsible for management of the [redacted] HPC Server composed 544 total compute cores and an aggregated memory of 2.9TB. The system is composed of (15) PowerEdge R430 1U systems (128 GB RAM each), (1) PowerEdge R830 (1024 GB RAM) and a high-speed 10GbE interconnect for intra-node communication. The HPCC also houses 832 TB RAW storage capacity.

EQUIPMENT

McCluskey Lab. The McCluskey lab occupies approximately 1500 sq. ft. in CA-3059. The lab is equipped with two complete neurophysiology rigs including: Zeiss OPMI surgical microscope, Powerlab hardware and software, Grass amplifier, P511 power source, Hum Bug 60 Hz noise reducer, integrator, oscilloscope, TMC air table with cage, and Dell Latitude laptops with each rig. We also have a live fluorescence imaging system with Olympus BX51WI microscope, Dell precision T3500 computer with wide monitor, MetaFluor software (Universal Imaging), Warner stimulus delivery system and multiple Warner RC-50 modified epithelial imaging chambers with custom 1 mm insert. Another image analysis system is equipped with Olympus BX50 microscope, digital cameras for fluorescent and brightfield microscopy (Roper Scientific), Dell Optiplex computer and MetaMorph software. We also have a Leica cryostat, BioTek Plate reader, gel electrophoresis equipment and thermal cyclers, standard laboratory benchtop and surgical equipment, a refrigerated centrifuge (Fisher 1R), -80º and -20ºC freezers and Dell desktop computers for personnel use in addition to those used with equipment. We have 24-hr access to Kodak gel documentation system, cell culture facilities (laminar flow hoods, incubators, inverted microscope) adjacent to PI's laboratory, and a QuantStudio 3 real-time PCR system (ThermoFisher).

Gan Laboratory. Dr. Gan's main laboratory occupies approximately 2,000 sq. ft. in the adjacent rooms CA3055 and CA3054A on the 3rd floor within the [redacted] The laboratory is fully equipped for tissue culture (Thermo Scientific biosafety cabinet/CO2 incubator), molecular biology and biochemistry (Thermo Scientific -80ºC freezer, Milli-Q IQ700 water purification system, NanoDrop One^{C} for DNA and RNA quantification, Eppendorf 5804 benchtop centrifuges, Eppendorf 5424 and 5424R microcentrifuges Bio-Rad QX200^{TM} Droplet Digital PCR, Bio-Rad CFX qPCR system, Bio-Rad C1000 thermal cyclers, Diagenode Bioruptor sonicator, Bio-Rad Gel Doc XR+, Bio-Rad semi-dry blotter, Eppendorf Vacufuge Plus, bacterial incubators and shakers), immunohistochemistry and in situ hybridization (Cryostar NX50 cryostat, Hybaid rotating/shaking ovens), embryo/tissue dissection stations (Nikon SMZ1500 and SMZ-U stereoscopes), bright light/DIC/pifluorescent imaging stations (Nikon TE2000U inverted microscope with DS-Ri2 camera and Zeiss Lumar V12 stereoscope with AxioCam camera), NEPAGENE NEPA21 in vitro & in vivo electroporator for cultured cells or explants.

Department of Neuroscience & Regenerative Medicine (NDRM) Facilities

Transgenic and Genome Editing Core Facility [redacted]
The Genome Editing Core is a full-service facility supported by [redacted], [redacted] and user fees. The Core's mission is to provide timely and cost-effective services in all phases of transgenic mouse production by pronuclear microinjection into zygotes, gene targeting in embryonic stem cells (ESCs), and gene editing using CRISPR/Cas9 approach to the research community at Augusta University as well as other academic institutions and for-profit companies. The Core also provides services on preserving your valuable lines by cryopreservation of sperms and embryos, and resurrecting mouse lines using in vitro fertilization (IVF) or embryo transfer. Additional services include rederivation to create pathogen-free mouse lines, developing molecular reagents for genetic engineering, full colony management services, and expert advice on mouse genetics and breeding schemes.

Microscopy [redacted] The imaging core is equipped with a Nikon A1R MP+ Multiphoton/Confocal Microscope with four high-sensitivity detectors for multiphoton imaging, seven visible laser lines for confocal imaging, a MaiTai eHP DS ultrafast laser, full electrophysiology recording capabilities, fast imaging: 420 frames/sec maximum speed, high-speed piezo-drive focus motor, simultaneous photostimulation and imaging, and a best-in-class two-photon objective. Another Nikon A1R MP+ Multiphoton/Confocal Microscrope has four visible laser lines for confocal imaging and the same capabilities as A1R system above except for electrophysiology. The facility also includes a Nikon C1 Confocal with three visible laser lines and an imaging workstation with deconvolution software (NIS Elements).

Light-Sheet Microscopy Core. [redacted] *Departmental facility recently became operational and personnel available for training in August, 2021*
The [redacted] operates an Imaging Core Facility with a newly installed Lavision Ultramicroscope II light-sheet microscope (Miltenyi Biotec). The Ultramicroscope II has an infrared laser and a second tunable laser for acquisition of up to three excitation channels (488/560/633). This system is capable of imaging samples up to 8x8cm in aqueous or organic media in Zoom Body or Super Plan

configuration. In the Super Plan configuration with the 12x objective, this system can acquire images with a maximal XY resolution of 0.217 microns/pixel. Resolution in the z-axis is determined by sample size, clearing efficacy, and laser configuration, but optical sections can be acquired at user-specified intervals for subsequent registration, segmentation, and high-throughput morphometric analysis. File size for tiled image acquisitions can reach 500GB per sample, and Augusta University provides unlimited cloud storage on an internal secure ftp site for data transfer and backup purposes. The departmental imaging facility also has two analysis workstations; one with Neurolucida 360 Studio for automated neuron tracing, dendritic spine and synapse detection, and a second with NeuroInfo software for registration onto the Allen Brain Atlas, automated segmentation, and cell detection (both from Microbrightfield).

Biostatistics and Bioinformatics Core [redacted] The Biostatistics and Bioinformatics Core is a shared departmental resource for biostatistics and bioinformatics consulting and support ranging from project design to data analysis. It is a critical component of the research infrastructure in the [redacted] Statistical analyses provided include statistical design of biomedical, translational and clinical studies, including sample size and power analysis; testing of assumptions of statistical tests; applying regression-based models to identify factors associated with disease outcomes; development of novel statistical methods and models; and bioinformatics analysis for Bioinformatics Analysis RNA-seq, microarray, ChIP-seq, DNA methylation, and whole genome sequencing.

Viral Vector Core [redacted] The mission of the Viral Vector Core is to provide technological resources to [redacted] faculty members interested in the use of viral based vectors for basic research and preclinical applications**.** The core is supported by the DNRM Research Enrichment Funds. The vector core focuses on producing lentiviral, gammaretroviral and recombinant adeno-associated viral (rAAV) particles for transgene expression, gene silencing and gene editing in either dividing or non-dividing cells. The following viral vectors are available from the core: lentiviral vectors, adeno-associated viral vectors and gammaretroviral vectors.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: LYNNETTE Middle Name Marie Last Name*: MCCLUSKEY Suffix:

Position/Title*: Professor
Organization Name*: [redacted]
Department: [redacted]
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., [redacted]

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

Degree Type: PHD Degree Year: 1996

Attach Biographical Sketch*: File Name: McCluskey_Biosketch_R21_Aug2021.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-20-195 . Received Date: 2021-08-05T18:21:52.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Lin Middle Name Last Name*: Gan Suffix:

Position/Title*: Professor
Organization Name*: [redacted]
Department: [redacted]d
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: ligan@augusta.edu

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

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

Degree Type: PhD Degree Year: 1992, 1985

Attach Biographical Sketch*: File Name: 2021.8_Gan_NIH-Bio_ACE2_FINAL.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Daniel Middle Name F. Last Name*: Linder Suffix:

Position/Title*: Assistant Professor
Organization Name*: [redacted]
Department: [redacted]
Division: [redacted]gia
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,MS,BS Degree Year: 2013,2008,2006

Attach Biographical Sketch*: File Name: Linder_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Camille Middle Name Tessitore Last Name*: King Suffix:

Position/Title*: [redacted]
Organization Name*: [redacted]
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]1

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: PHD Degree Year: 1990

Attach Biographical Sketch*: File Name: biosketch-C_King2.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Michael Middle Name Stephen Last Name*: King Suffix:

Position/Title*: [redacted]
Organization Name*: [redacted]
Department: [redacted]
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

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

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: PHD,AB Degree Year: 1992,1986

Attach Biographical Sketch*: File Name: biosketch-M_King2.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Xin-Yun Middle Name Last Name*: Lu Suffix:

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: MD,PHD Degree Year: 1987,1998

Attach Biographical Sketch*: File Name: LU_Biosketch_July_2021.pdf

Attach Current & Pending Support: File Name:

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: Lynnette Phillips McCluskey

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Florida State University, Tallahassee, FL	B.A.	12/1990	Psychology / Biology
University of Virginia, Charlottesville, VA	M.S.	06/1995	Psychobiology
University of Virginia, Charlottesville, VA	Ph.D.	04/1996	Psychobiology
Brigham & Women's Hospital/Harvard Medical School, Boston, MA	Postdoc	09/2000	Neuroimmunology

A. Personal Statement

My long-standing interest is in taste-immune interactions. The main focus of my research group is on the impact of cytokines and leukocytes on regenerating taste buds. The taste system is inherently plastic, but mechanisms responsible for taste bud and nerve regeneration and the recovery of taste function are poorly understood. Insights to immune regulation of regeneration are needed to develop therapies to recover taste following inflammation and neural injury. My laboratory has recently found that IL-1R signaling in infiltrating leukocytes and taste buds is needed to restore normal neurophysiological taste responses and fully regenerate taste buds. As taste deficits emerged as a major symptom of COVID-19 I began studies on the SARS-CoV-2 receptor, ACE2, in mice and collaborative work on COVID-19 in patients based on my expertise in the taste-immune axis. My role is to advise a team of neurologists on taste and smell testing to determine whether long-term chemosensory deficits emerge in COVID-19 positive patients, half of whom are African-American and at greater risk of COVID-19. In a supplement to Dr. David Hess' R01 we test the hypothesis that there will be clinical, racial and genetic predictors of the occurrence and severity of neurological sequelae. In the current R21 application my team proposes to study inflammation and SARS-CoV-2 spike protein challenge in novel mouse models developed by Dr. Gan to shed light on the molecular, cellular, and neurophysiological role of ACE2 in the taste system. Our group is well-positioned to contribute fundamental insights to taste loss due to SARS-CoV-2.

Complete list of published works in My Bibliography *(Note: the PI has published under the names Lynnette McCluskey and Lynnette Phillips)*
https://www.ncbi.nlm.nih.gov/myncbi/lynnette.mccluskey.1/bibliography/public/

B. Positions and Honors

Professional Positions

2001-2005 Assistant Professor, Dept. of Physiology, Medical College of Georgia, Augusta, Georgia
2005-2006 Assistant Professor, Dept. of Neuroscience & Regenerative Medicine, Medical College of Georgia, Augusta, Georgia
2005-2020 Associate Professor (tenured), Dept. of Neuroscience & Regenerative Medicine, Medical College of Georgia at Augusta University, Augusta, Georgia

2020-present Professor, Dept. of Neuroscience & Regenerative Medicine, Medical College of Georgia at Augusta University, Augusta, Georgia

2009-2020 Director, Graduate Program in Molecular Medicine
2020-present Director, Graduate Program in Neuroscience

Honors

1993, 1995 Travel Award, Association for Chemoreception Sciences (AChemS)
1996-1998 NRSA Fellowship
1996-1999 National Multiple Sclerosis Society Fellowship
1998 Dale McFarlin Travel Award, National Multiple Sclerosis Society (one of two awarded yearly)
2006 Recipient, Ajinomoto Young Investigator in Gustation Award, AChemS
2014 Distinguished Service Award, The Graduate School, MCG
2015 Distinguished Teacher Award, The Graduate School, MCG

Other Professional Activities

2001-present AChemS (Student member from 1992-1996)
2001-present Reviewer for journals including: *J Neurophysiol*, *Exp Neurol, Neurosci, Am J Physiol, Chem Senses, Neurosci Lett, Dev Dynamics, NeuroImmunoModulation, J Lipid Res, Dev Biol, PLOS One, J Neurosci, Sci Rep, J Gene Dev, J Pharmacol Exp Therap, Genesis, Nutrients, ACS Pharm Translational Sci, Int J Mol Sci, Nutrients, ACS Pharm Transl Sci, Cell Prolif*

2008-2010 Councilor (Executive Committee), AChemS
2009 Reviewer for NIAID Special emphasis panel “Immune Defense Mechanisms at the Mucosa”
2011 Reviewer for Thomas F. and Kate Miller Jeffress Memorial Trust to support research in Virginia
2014 *Ad hoc* reviewer, Neural Systems Cluster, Integrative Organismal Systems Division, National Science Foundation, April 2014
2013-2019 Member, MCG Institutional Animal Care & Use Committee (IACUC); Vice-Chair 2016-2019
2017-2020 Program Committee member, AChemS
2017 Symposium Co-Chair, “Emerging mechanisms for sensory-immune interactions”, AChemS, Bonita Springs, FL 2017
2018-2019 NIH *Ad hoc* Reviewer, Special Emphasis Panel/Scientific Review Group 2019/01 ZDC1 SRB-R, 10/12/2018, 10/24/2018, 2/21/2019, 6/25/2019, 7/1/2019
2019 NIH *Ad hoc* Reviewer, Scientific Review Group Clinical and Integrative Diabetes and Obesity (CIDO) 10/2019
2020 NIH *Ad hoc* Reviewer, Scientific Review Group Chemosensory Systems (CSS), 10/15/2020
2020 NIH *Ad hoc* Reviewer, Special Emphasis panel, COVID-19 U01 applications (NIDCD) ZRG1-IFCN-B-51, 10/16/20
2020 Kansas IDeA Network of Biomedical Research Excellence (K-INBRE) reviewer, Bridging Grant Award
2021 NIH *Ad hoc* Reviewer, Special Emphasis panel, ZRG1 IFCN-J 02 M and ZRG1 IFCN-U (07) 07/14/2021

C. Contributions to Science

1. My undergraduate training at Florida State University included research in taste and feeding behavior with Dr. James Smith, Robert O. Lawton Distinguished Professor (now Emeritus). This experience combined with strong didactic and laboratory training in sensory systems fostered my career-long interest in chemosensation. I then trained in taste neurophysiology with Dr. David Hill at the University of Virginia for graduate studies. As an extension of Dave’s formative studies demonstrating developmental taste plasticity, I became interested in adult plasticity triggered by taste bud and neural injury. We found that altering the dietary environment by depleting sodium induced neurophysiological changes in regenerated taste buds as during development. Surprisingly, the function of intact taste buds on the opposite side of the tongue was also affected by neighboring nerve injury. I hypothesized that diet-induced immunosuppression was responsible for contralateral functional deficits. Indeed, by stimulating immune function with lipopolysaccharide (LPS) normal

neural responses were restored. This sparked my >20 year interest in taste-immune interactions which is the focus of the current application. Once I established my own laboratory at the Medical College of Georgia, the Hill lab and my team collaborated to show that exogenous aldosterone, like ACE2 a member of the renin-angiotensin system (RAS) 2, impacts both taste function and macrophage responses to nerve injury. In this R21 proposal Dr. Gan and I test whether deletion of Ace2 in the lingual epithelium will also alter taste responses and local inflammatory responses.

a. Hill, DL and LM Phillips (1994) Functional plasticity of regenerated and intact taste receptors in adult rats unmasked by dietary sodium restriction. *J Neurosci*, 14: 2904-2910.

b. Phillips, LM and DL Hill (1996) Novel regulation of peripheral gustatory function by the immune system. *Am J Physiol,* 271: R857-R862.

c. Guagliardo N, West K, McCluskey L, and DL Hill (2009) Attenuation of peripheral salt taste responses and local immune function contralateral to gustatory nerve injury: effects of aldosterone. *Am J Physiol.* 297: R1103-R1110. PMID: 19675282

2. My postdoctoral training with Dr. Lois Lampson at Brigham & Women's Hospital / Harvard Medical School provided intensive studies in neuroimmunology. Dr. Lampson's laboratory was part of the Center for Neurologic Disease which focuses on the immune mechanisms mediating damage in multiple sclerosis, Alzheimer's disease and brain tumor. My training there, supported by NIH and the National Multiple Sclerosis Society, focused on the effect of local brain microenvironments on T cell and microglial responses to cytokines. During this period I added immunological and CNS neuroanatomical techniques to my background in sensory neurophysiology.

a. Phillips, LM and LA Lampson (1999) Site-specific control of T cell traffic in the brain: T cell entry to brainstem vs. hippocampus after local injection of IFN-γ. *J Neuroimmunol*, 96: 218-227.

b. Phillips, LM, PJ Simon, and LA Lampson (1999) Site-specific immune regulation in the brain: differential modulation of major histocompatibility complex (MHC) proteins in brainstem vs. hippocampus. *J Comp Neurol*, 405: 322-333.

c. McCluskey, LP and LA Lampson (2000) Local neurochemicals and site-specific immune regulation in the CNS. *J Neuropathol Exp Neurol*, 59: 177-187.

d. McCluskey, LP and LA Lampson (2001) Local immune regulation in the CNS by substance P and glutamate. *J Neuroimmunol, 116: 136-146.*

3. My research program was an early contributor to the subfield of taste-immune interactions. My group has shown the functional significance of neutrophil, macrophage, cytokine and chemokine responses in the normal and injured taste system. For example, neutrophils and macrophages have opposing, dynamic effects on taste function. Neutrophils responding to nerve injury or inflammation are detrimental to sodium taste responses, while macrophages are beneficial for the return of normal function. We have also studied the effects of cytokines released by leukocytes on taste function and determined that IL-1 and TNF have opposite effects on sodium flux in polarized taste buds, with a net increase in neural sodium sensitivity. These studies are important for our understanding of taste changes that impact nutrition during infection, injury, and local and systemic inflammation. Our work has stimulated interest into the normal and pathological roles of inflammation in the taste system. Recently we have determined that interleukin (IL)-receptor signaling is necessary for regenerating taste buds and recovering normal neurophysiological taste function after chorda tympani nerve sectioning in mice. We are currently testing genetic models of interleukin (IL)-1 receptor deletion in leukocytes or taste buds to determine when and where IL-1 signaling is needed to restore taste function after nerve mice.

a. McCluskey, LP (2004) Upregulation of activated macrophages in response to degeneration in the taste system: effects of dietary sodium restriction. *J Comp Neurol,* 479: 436-55. PMID: 15389612

b. Shi L, He L, Sarvepalli P, and McCluskey LP (2011) A functional role for IL-1 in the injured peripheral taste system. *J Neurosci Res* 90: 816-830. PMID: 22213141.

c. He L, Yadgarov A, Sharif S, and McCluskey LP (2012) Aging profoundly delays functional recovery from gustatory nerve injury. *Neuroscience* 3:208-18. PMID: 22387273

d. Kumarhia D and McCluskey LP (2016) Inflammatory stimuli acutely modulate peripheral taste function. *J Neurophysiol* 115: 2965-75. PMID: 27009163

4. Animal and human studies link gut inflammation and infection with taste deficits through unknown mechanisms. In collaborative studies with Dr. David Pittman we used a mouse model to test the hypothesis that gut inflammation alters taste function and behavior. Oral gavage with lipopolysaccharide (LPS), a proinflammatory stimulus derived from gram-negative bacteria, acutely reduces neural and behavioral responses to sweet stimuli through toll-like receptor (Tlr)-4 signaling. Chronic LPS gavage prolongs these deficits in sweet responses and also decreases salt taste responses. Taste changes relevant to health and nutrition are thus triggered by localized gut inflammation. In a collaboration with MCG colleague, Dr. Ruth Harris, we observed similar changes in neural taste responses to sweet and salt in rats chronically ingesting liquid sucrose which parallels human overconsumption of sweet stimuli. Current work is aimed at identifying changes in taste function in mouse models of inflammatory bowel disease.

a. Pittman DW, Dong G, Brantly AM, He L, Nelson TS, Kogan S, Powell J and McCluskey LP (2020) Behavioral and neurophysiological taste responses to sweet and salt are diminished in a model of subclinical intestinal inflammation. *Sci Rep* 10(1): 17611. PMID: 33077838.

b. McCluskey LP, He L, Dong G, Harris R. (2020) Chronic exposure to liquid sucrose and dry sucrose diet have differential effects on peripheral taste responses in female rats. *Appetite*. 2020 Feb 1;145:104499. PMID: 31669578

c. Zhu X, He L, and McCluskey LP (2014) Ingestion of lipopolysaccharide inhibits peripheral taste responses to sucrose in mice. *Neuroscience* 258: 47-61. PMID: 2421598.

5. Taste and smell deficits are prominent symptoms of COVID-19 viral infection caused by SARS-CoV-2. Though most patients recover taste and smell sensation a subset of COVID-19 patients experience longer-term sensory deficits detrimental to health and nutrition. The number of people with chronic chemosensory deficits is likely significant given the massive scale of the current pandemic. My interest in taste-immune interactions and ~25 years in chemosensory research complements the team of Medical College of Georgia neurologists testing neurologic symptoms of COVID-19.

Hess DC, Rutkowski E, Morgan J and L McCluskey (2020) COVID-19 and neurological symptoms: Is the COVID-CoV-2 virus neurotropic? *Cond Med* 3(5): 241-245. PMID: 34136764

D. Additional Information: Research Support

Current Research Support

R01 DC016668 National Institutes of Health (NIDCD) 5/1/18-4/30/23
McCluskey (PI)
Cytokines in the regenerating taste system
The goal of this project is to determine how IL-1 signals through its receptor to rebuild taste buds and restore taste function after injury.

Completed Research Support (last 3 years)

R01 3R01NS112511-01A1S1 National Institutes of Health (NINDS) 08/01/2020 – 01/31/2021
Hess (PI) McCluskey (Co-I)
COVID-19 neurological Georgia cohort study
This supplement supports studies to identify factors associated with neurological symptoms including chemosensory deficits in COVID-19 patients.

PSRP 000102 Pilot Study Research Program Grant, MCG. 11/1/17-10/31/18
McCluskey (PI)
Taste changes in a mouse model of inflammatory bowel disease

This project supported preliminary studies for the submission of an R01 investigating taste changes in mice with chronic DSS-induced colitis.

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Gan, Lin

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Zhongshan University, Guangzhou, China	B.S.	07/1985	Biochemistry
University of Texas Graduate School of Biomedical Sciences, Houston, TX	Ph.D.	12/1992	Biochemistry and Molecular Biology
U.T. M.D. Anderson Cancer Center, Houston, TX	Postdoc.	12/1995	Developmental Biology
Fundamental Issues in Vision Research-NEI sponsored summer training program at MBL	-	08/1994	Vision Research

A. Personal Statement

The long-term objective of my research is to understand the molecular mechanisms underlying neuronal differentiation and disease, especially the function of transcription factors (TFs) in sensory organs such as the mammalian inner ear and retina. As a graduate student, I first became interested in the roles of transcription factors in embryonic development. My work on the transcription factors has contributed significantly to our understanding of the molecular mechanisms governing neural development in the retina and inner ear, such as the *Atoh7→Sox4/Sox11→Isl1/Pou4f2* regulatory pathway in the development of retinal ganglion cells, and the roles of *Pou4f3*, *Gata3*, and *Lmo4* in inner ear development. In recent years, my work on subtype specification of retinal neurons has revealed the roles of several transcription factors and the underlying molecular mechanism specifying neuronal subtypes during retinogenesis. Additionally, my research has made available to the research community over 100 genetically modified mouse strains and other reagents, particularly tissue-specific Cre deleter mouse strains such as *Itga8-CreERT2*, *Bhlhb5-Cre, Atoh7-Cre, Gfi1-Cre, Gfi1-2A-CreERT2*, *Sox2-2A-CreERT2*, and *Atoh1-Cre*.

In addition, I am an expert in mouse genetics and related transgenic/knockout technologies with over 28 years of experience in transgenic and knockout mouse generation and analysis. I have founded and directed transgenic/knockout facilities at The University of Texas M.D. Anderson Cancer Center (1996-1999), University of Rochester (2006-2019), and Augusta University Medical College of Georgia (2019-present). Throughout my research career, I have successfully developed, improved, and incorporated new transgenic, gene targeting in ES cells, and CRISPR/Cas9 techniques to address diverse scientific challenges. Over 1,000 knockout/knock-in/conditional knockout mouse models have been created by me or under my supervision, including about 500 by CRISPR/Cas9 method in the past 6 years.

Specific for this application, I will assist with the generation of three novel *Ace2* strains and will make my expertise available to project design, *Ace2*-expressing cell lineage tracing, *Ace2* cKO phenotypic analysis, breeding schemes, and genotyping strategies.

Ongoing and recently completed projects that I would like to highlight include:

Ongoing Research Support

1R01EY026614-01 02/18/2016-02/28/2022 (NCE)
NIH/NEI

Title of Project: “The Roles of LIM-Homeodomain Transcription Factors in Retinal Development”
The major goal of this proposal is to identify the role of LHX9’s role in the specification of retinal amacrine neuronal subtype.
Role: PI

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

10/2019- present	Professor, Department of Neuroscience and Regenerative Medicine, Augusta University Medical College of Georgia
10/2019-present	Director of Genome Editing Core, Augusta University Medical College of Georgia
10/2019- present	GRA Eminent Scholars, Georgia Research Alliance
7/2009-9/2019	Professor of Ophthalmology, Neurobiology and Anatomy, Center for Neural Development and Diseases, and Center for Visual Sciences, University of Rochester
7/2009-9/2019	Dean’s Professor (endowment/chair), University of Rochester Medical Center
7/2009-9/2012	Director of Research, Flaum Eye Institute, University of Rochester Medical Center
9/2006-9/2019	Director, Mouse Genome Editing Resource (formerly The Transgenic and Gene Targeting Core), University of Rochester Medical Center
6/2005-6/2009	Associate Professor, Dept. of Ophthalmology, Department of Neurobiology and Anatomy, Center for Visual Sciences, University of Rochester Medical Center
9/1999-5/2005	Assistant Professor, Dept. of Neurobiology and Anatomy, Center for Aging and Developmental Biology, University of Rochester Medical Center
9/1996-8/1999	Assistant Professor, Dept. of Biochem. & Mol. Biol., The University of Texas M.D. Anderson Cancer Center.
9/1996-8/1999	Founding Director of Gene Targeting (Knockout) Core, The University of Texas M.D. Anderson Cancer Center.
1/1996-8/1996	Instructor, Dept. of Biochem. & Mol. Biol., U.T. M.D. Anderson Cancer Center.

Selected Memberships, Editorial Boards and Advisory Committees

2021.2-2021.6	Standing Member of the Biology and Development of the Eye (BDE) Study Section, NIH
2017.7-2020.11	Standing Member of the Biology of the Visual System (BVS) Study Section, NIH
2010-2015	Editorial Board: Journal of Biological Chemistry
2007-present	Member, Association for Research in Otolaryngology (ARO)
2006-present:	Ad hoc Member of the following NIH Study Sections: NDPR (Neural Differentiation, Plasticity, and Regeneration) Study Section (2006.2), NIH BDPE (Biology and Diseases of the Posterior Eye) Study Section (2008.6, 2009.2, 2009.6), NIH ZRG1 CB-G 02 S (Retinopathy Models and Therapy) Study Section (2009.6), NIH NCF (Neurogenesis and Cell Fate) Study Section (2009.10), NIH ZRG1 CB-G 90 (Retinopathy Models and Therapy) Study Section (2010.2), ZRG1-IFCN-B-02M (Auditory Neuroscience) Study Section (2010.10), ZRG1 BDPE-N (09) (Biology and Diseases of the Posterior Eye) Study Section (2011.9), Neurodifferentiation, Plasticity, Regeneration and Rhythmicity (NDPR) study section (2012.2), Special Emphasis Panel/Scientific Review Group 2013/01 ZDC1 SRB-R (38) (2012.10), Special Emphasis Panel/Pain and Hearing ZRG1-IFCN-B-02M (2013.6), Neurodifferentiation, Plasticity, Regeneration and Rhythmicity (NDPR) study section (2014.2).
2005-present	Member, Society for Neuroscience (SfN)
2003-present	Life Member, Society of Chinese Bioscientists in America (SCBA)
2001-present	Life Member, The Chinese Biological Investigators Society (formerly Ray Wu Society) Life Member
1999-present	Member, The American Association for the Advancement of Science (AAAS)
1998-present	Member, Association for Research in Vision and Ophthalmology (ARVO)

Honors

2010	Research to Prevent Blindness Senior Scientific Investigator Award
1999-2003	Rosanne H. Silbermann Fellow
1985	CUSBEA (China-United States Biochemistry and Molecular Biology Examination and Administration, aka Ray Wu Program) Scholar, Ministry of Education, Beijing, China

C. Contributions to Science

1. Development of retinal ganglion cells

My early publications focused on the roles of transcription factors in retinal development, particularly the development of retinal ganglion cells (RGCs). I have shown that ATOH7 determines the RGC-competence of retinal progenitors and that POU4F2 and ISL1 are the key RGC differentiation factors acting synergistically to regulating the differentiation and survival of RGCs. Furthermore, we have demonstrated that SOX4 and SOX11 act upstream of POU4F2/ISL1 and downstream of ATOH7 in this process. Thus, these TFs constitute the ATOH7→SOX4/SOX11→ISL1/POU4F2 regulatory pathway. Taken together, my work has contributed significantly to our currently understanding of the molecular mechanisms governing retinal ganglion cell development.

a. **Gan, L.**, Xiang, M., Zhou, L., Wagner, D.S., Klein, W.H., and Nathans, J. (1996). POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. ***Proc. Natl. Acad. Sci****.* 93, 3920-3925.

b. Wang, S.W., Kim, B.S., Ding, K., Wang, H., Sun, D., Johnson, R.L, Klein, W.H. and **Gan, L.** (2001). Requirement of math5 in the development of retinal ganglion cells. ***Genes & Dev.*** **15**, 24-29.

c. Pan, L., Deng, D., Xie, X., and **Gan, L.** (2008). ISL1 and BRN3B co-regulate the differentiation of murine retinal ganglion cells. ***Development*** 135, 1981-1990. PMCID: PMC2758274. (**cover photograph**)

d. Jiang, Y., Ding, Q., Xie, X., Libby, R., Lefebvre, V., and **Gan, L.** (2013). Transcription factors SOX4 and SOX11 function redundantly to regulate the development of retinal ganglion cells. ***J. Biol. Chem.*** 288, 18429-18438. PMCID: PMC3689985.

2. Specification of neuronal subtypes in the retina

Besides RGC development, my work on subtype specification of retinal neurons, along with the studies by other investigators, has begun to reveal the underlying molecular mechanism specifying neuronal subtypes in the retina, which unique combinations of transcription factors determine specific retinal subtypes.

a. Feng, L., Xie, X., Joshi, P., Yang, Z., Shibasaki, K., Chow, R., and **Gan, L.** (2006). Requirement for Bhlhb5 in the specification of amacrine and cone bipolar subtypes in mouse retina. ***Development*** **133**, 4815-4825. PMID: 17092954. (**cover photograph**)

b. Elshatory, Y., Deng, M., Xie, X., Barlow, R.B., and **Gan, L.** (2007). Islet-1 controls the differentiation of retinal bipolar and cholinergic amacrine cells. ***J. Neurosci.*** 27:12707-12720. PMID: 18003851.

c. Ding, Q., Chen, H., Xie, X., Libby, R., Tian, N., and **Gan, L.** (2009). BARHL2 differentially regulates the development of retinal amacrince and ganglion neurons. ***J. Neurosci.*** 29, 3992– 4003. PMCID: PMC2756297. (**cover photograph)**

d. Dong, X., Yang, H., Zhou, X., Xie, X., Yu, D., Guo, L., Xu, M., Zhang, W., Liang, G., and **Gan, L**. (2020). LIM-Homeodomain Transcription Factor LHX4 Is Required for the Differentiation of Retinal Rod Bipolar Cells and OFF-Cone Bipolar Subtypes. ***Cell Rep.*** 2020 Sep 15;32(11):108144. doi: 10.1016/j.celrep.2020.108144. PubMed PMID: 32937137.

3. Roles of transcription factors in the inner ear

I was first attracted to the development of inner ear during my postdoctoral training and demonstrated POU4F3 (Brn3c) as an essential TF in the development and survival of inner ear hair cells (***Proc. Natl. Acad. Sci.***, 94:9445-9450). In the recent years, I have focused on a group of functionally associated TFs of GATA, LMO, and ISL family proteins, which are uniquely expressed in the cochlear epithelium prior to and during the formation of prosensory domain. I have begun to show that these TFs positively or negatively regulate the formation of cochlear sensory domain. This body of work reveals a novel network of TFs that function upstream of SOX2, a master regulator of prosensory specification, to specify the prosensory domain as well as non-sensory regions in the cochlea.

a. Deng, M., Pan, L., Xie, X., and **Gan, L.** (2010). Requirement for *Lmo4* in the vestibular morphogenesis of mouse inner ear. ***Dev. Biol.*** 338, 38–49. PMCID: PMC2812651. **(cover photograph)**

b. Luo, X., Deng, M., Xie, X., Huang, L., Wang, H., Jiang, L., Liang, G., Hu, F., Tieu, R., Chen, R., and **Gan, L.** (2013). GATA3 controls the specification of prosensory domain and neuronal survival in the mouse cochlea. ***Hum. Mol. Genet.*** 22, 3609-3623. PMCID: PMC3749857. (**cover photograph**)

c. Deng, M., Luo, X., Pan, L., Yang, H., Xie, X., Liang, G., Huang, L., Hu, F., Kiernan, A., and **Gan, L.** (2014). LMO4 functions as a negative regulator of sensory organ formation in the mammalian cochlea. ***J. Neurosci.*** 34, 10072-7. PMCID: PMC4107398. **(Selected for F1000Prime)**

d. Xu, J., Yu, D., Dong, X., Xie, X., Xu, M., Guo, L., Huang, L., Tang, Q., and **Gan, L.** (2021). GATA3 maintains the quiescent state of cochlear supporting cells by regulating p27kip1. ***Sci. Rep.*** 11:15779.

4. Other contributions to the neuroscience field

While my major research focus has always been in the development of retina and inner ear, my study using mouse genetic models often leads to observations of great significance in the development and disease of other nervous systems. I have been able to recognize the importance of these findings and to elucidate the underlying mechanisms by myself as well as in collaboration with experts in the related field. Often, these studies are published in high-impact journals and demonstrate my commitment to broad scientific collaborations and our ability to initiate and carry to fruition research at high level in research fields other than my specialty in retina and inner ear.

a. Elshatory, Y. and **Gan, L.** (2008). The LIM-homeobox gene Islet-1 is required for the development of restricted forebrain cholinergic neurons. ***J. Neurosci.*** 28:3291–3297. PMCID: PMC2786914.

b. Joshi, P.S., Molyneaux, B.J., Feng, L., Xie, X., Macklis, J., and **Gan, L.** (2008). Bhlhb5 Regulates the Post-Mitotic Acquisition of Area Identities in Layers II-V of the Developing Neocortex. ***Neuron*** 60, 258–272. PMCID: PMC2643370**.**

c. Ding, Q. Joshi, P.S., Xie, Z., Xiang, M., and **Gan, L.** (2012). BARHL2 transcription factor regulates the ipsilateral/contralateral subtype divergence in postmitotic dI1 neurons of the developing spinal cord. ***Proc. Natl. Sci. Acad., USA***. 109: 1566-1571. PMCID: PMC3277160.

d. Luo, X., Li, M., Huang, L., Steinberg, S., Mattheisen, M., Donohoe, G., Shi, Y., Chen, C., Yue, W., Alkelai, A., Lerer, B., Li, Z., Yi, Q., Rietschel, M., Cichon, S., Collier, D.A., Tosato, S., Suvisaari, J., Rujescu, D., Golimbet, V., Silagadze, T., Durmishi, N., Milovancevic, M.P., Stefansson, H., Schulze, T.G., Nöthen, M.M., Chen, C., Lyne, R., Morris, D.W., Gill, M., Corvin, A., Zhang, D., Dong, Q., Moyzis, R.K., Sigurdsson, E., Stefansson, K., Hu, F., MooDS SCZ Consortium, Su, B., and **Gan, L.** (2014). Convergent lines of evidence support *CAMKK*2 as a schizophrenia susceptibility gene. ***Molecular Psychiatry.*** 19, 774-83. PMID: 23958956. **(Selected for F1000Prime).**

Complete List of 110 Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/lin.gan.1/bibliography/40475286/public/?sortby=pubDate&sdirection=descending

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

BIOGRAPHICAL SKETCH

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

NAME: Linder, Daniel Frederick

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

POSITION TITLE: Assistant Professor of Biostatistics and Data Science

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
Georgia Southern University, Statesboro, Georgia	BS	07/2006	Mathematics
Georgia Southern University, Statesboro, Georgia	MS	05/2008	Mathematics
AU (Medical College of Georgia), Augusta, Georgia	PhD	07/2013	Biostatistics

A. Personal Statement

My background is in mathematics and biostatistics, and I have specific research interests in developing Bayesian statistical methods for stochastic reaction networks and more generally in developing Bayesian machine learning methods for high-dimensional problems, key research areas for this application. I have been involved in several funded projects that involved developing new statistical methodology for reaction networks, big biomedical data, longitudinal data, and cluster analysis, all of which were eventually published in peer reviewed journals. In May of 2016, I joined the faculty in the Department of Population Health Sciences in the Medical College of Georgia at Augusta University. As a faculty member I have directed a doctoral dissertation on Bayesian modeling using global-local shrinkage rules for inferring gene regulatory networks, and I have also directed another PhD dissertation on the topic of statistical methods in stochastic reaction networks. Further, I am currently directing a PhD dissertation in which my student has proposed efficient Bayesian modeling strategies that link patient phenotypes to genotypes. As part of an Early Career Award from the Mathematical Biosciences Institute (MBI) at Ohio State University, I have worked with colleagues on developing survival dynamical models to model the spread of infectious diseases on networks. Since joining the department, I have developed collaboration with several clinical and basic sciences researchers at Augusta, and I am currently a collaborator on Dr. Lynnette McCluskey's R01. Part of this work is to understand the mechanisms of taste bud regeneration and the role that cytokine networks and genes play in the pro- and anti-regenerative process, with a goal of translating this work to help those with tongue injury repair taste. We currently have two manuscripts in preparation related to this work. For the current project, I will work closely with Dr. McCluskey and her team to investigate the role of the SARS-CoV-2 receptor, ACE2, in the taste system.

B. Positions and Honors

Positions and Employment

2012-2013 Visiting Assistant Professor of Biostatistics, Georgia Southern University (GSU), Statesboro, Georgia
2013-2016 Assistant Professor of Biostatistics, GSU
2016-Present Assistant Professor of Biostatistics and Data Science, Medical College of Georgia, Augusta University

Other Experience and Professional Memberships

2013-Present Eastern North American Region International Biometric Society, ENAR
2016-Present American Statistical Association

Honors

2006 Mathematical Association of America, 4th place in math jeopardy, Knoxville, TN
2006 Outstanding mathematics student award, GSU
2012 Travel award by Mathematical Biosciences Institute, CAMBAM-NIMBIOS Summer graduate program: Stochastics applied to biological systems, Ohio State University, Columbus, OH
2014 Modified Bayesian Lasso with L1 Loss, 1st place in applied statistics, APHA, New Orleans, LA
2015 Mathematical Biosciences Institute travel award: Treatment, Clinical Trials, Resistance Workshop
2016 Mathematical Biosciences Institute travel award: Topological, Geometric, and Statistical Techniques in Biological Data Analysis.

C. Contributions to Science

1. My main area of statistical research is at the cross-section of Bayesian statistics and computational biology. A large part of my work has focused on balancing model accuracy with computational feasibility. Some of the approaches I have developed have been based on both empirical and hierarchical Bayesian modeling. Specifically, my colleagues and I have developed highly tractable and efficient Bayesian techniques for inference in complex stochastic dynamical systems with a wide range of applications from systems biology to political science. My work on these mathematical models and their connection to Bayesian model selection methodology for high-dimensional data provides the requisite experience necessary to discover novel findings.
    1. **Linder, D.F.** & Rempala, G.A. (2020). Synthetic likelihood method for reaction network inference. Mathematical Methods in the Applied Sciences.
    2. **Linder, D.F.**, & Rempala, G.A. (2013). Algebraic statistical model for biochemical network dynamics inference. Journal of Coupled Systems and Multiscale Dynamics, 1(4),468–475.
    3. Panchal, V. & **Linder, D.F.** (2020). Reverse engineering gene networks using global-local shrinkage rules. Interface Focus.
    4. Volkening, A., **Linder, D.F.**, Porter, M.A., & Rempala, G.A. (2020). Forecasting elections using compartmental models of infections. In press at SIAM Review.

2. I have also worked on a series of publications developing efficient sampling strategies to maximize power in clinical studies. Efficient experimental design has become pertinent with the growing availability of high-throughput technologies, like next generation sequencing, where researchers are confronted with a need to collect measurements that are often costly and under constrained budgets. Part of my research activity has been on how to maximize the information content of a study by using carefully designed sampling strategies. The methodology developed in the publications below leverage the additional structure of order statistics to impart additional information into the study samples that are collected and measured. Thus, the collected samples will contain considerably more information than a corresponding simple random sample of the same size. Through my expertise in efficient sampling design, I will work with clinicians to design highly efficient and powerful clinical studies that have high power of success, while at the same time preserving valuable resources.
    1. **Linder, D.F.**, Samawi, H.M., Yu, L., Chatterjee, A., Huang, Y., & Vogel, R. (2015). On stratified bivariate ranked set sampling for regression estimators. Journal of Applied Statistics, 42(12), 15–23.
    2. **Linder, D.F.**, Yin, J., Rochani, H., Samawi, H., & Sethi, S. (2017). Increased Fisher's information for parameters of association in count regression via extreme ranks. Communications in Statistics (Theory and Methods). DOI: 10.1080/03610926.2017.1316859
    3. Samawi, H.M., Cai, J., **Linder, D.F.,** Rochani, H., Yin, J. (2018). A simpler approach for mediation anaylsis for dichotomous mediators in logistic regression. Journal of Statistical Computation and Simulation. DOI: 10.1080/00949655.2018.1426762

4. Samawi, H.M., Rochani, H., Yin, J., **Linder, D.F.,** Vogel,R. (2018). Notes on kernel-based mode estimation using more efficient sampling designs. Computational Statistics. DOI: 10.1007/s00180-017-0787-2

3. Further, I have collaborated with clinical and public health researchers on several projects ranging from the mining and analysis of large national databases to analyzing data from clinical trials, of which my role was as the principal biostatistician.
    1. Tarasenko, Y., **Linder, D.F.**, & Miller, E.E. (2018). Physical activity and mortality among 3+ year cancer survivors in the U.S. Cancer Causes and Control.
    2. Majeed, B., **Linder, D.F.**, Eissenberg, T., Tarasenko, Y.N., Smith, D., & Ashley, D.A. (2020). Cluster analysis of urinary biomarkers among US adults: Population Assessment of Tobacco and Health (PATH). Preventive Medicine.
    3. Park, Y.D., **Linder, D.F.**, Pope, J., Flamini, R.J., & Moretz, K., Diamond, M.P., Long, S.A. (2020). Long-term efficacy and safety of cannabidiol (CBD) in children with treatment-resistant epilepsy: Results from a state-based expanded access program. Epilepsy & Behavior.

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

Ongoing Research Support

- NIH 1R01 DC 016668 McCluskey, Lynnette (PI) 01/2018-12/2023
  Cytokines in the Regenerating Taste System
  The goal of this study is to examine the role of cytokines in the regeneration of taste.
  Role: Biostatistician

Completed Research Support

- The Department of Defense Chen, Jie (PI) 08/2019-07/2020
  Machine Learning
  Developed machine learning course for the United States military
  Role: Co-Investigator

- The Department of Defense Chen, Jie (PI) 08/2019-07/2020
  Theoretical Foundations of Machine Learning
  Developed a theoretical foundations course for the United States military
  Role: Co-Investigator

- Georgia Cannabidiol Study Park, Yong (PI) 06/2018-05/2020
  The Georgia Cannabidiol Study is an Intermediate Size Expanded Access clinical trial of the drug Epidiolex in pediatric seizure patients.
  Role: Biostatistician

- Early Career Award MBI/NSF Linder, Daniel (PI) 01/2018-12/2018
  The Ohio State University
  Role: Visiting research faculty

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

BIOGRAPHICAL SKETCH

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

NAME: Camille Tesstiore King, Ph.D.

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

POSITION TITLE: Chair & Professor of Psychology

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Virginia, Charlottesville, VA	B.A.	05/1985	Psychology
University of Virginia, Charlottesville, VA	M.A.	05/1988	Biological Psychology
University of Virginia, Charlottesville, VA	Ph.D.	05/1990	Biological Psychology
University of Virginia, Charlottesville, VA	Post-doc	12/1991	Central Taste Anatomy
University of Michigan Ann Arbor, MA	Post-doc	08/1993	Central Taste Anatomy
University of Florida, Gainesville, FL	Post-doc	08/1998	Behavioral Neuroscience

A. Personal Statement

My entire research career has been centered on the anatomy of the central gustatory system, chiefly on its development or regeneration, with a second focus on taste behaviors in adults in response to central gustatory manipulations. I have examined how the central termination fields of the taste nerves (using HRP-labeling) and the structures of first-order neurons (using Golgi-staining) are altered during development as a consequence of maternal sodium deprivation. For many years, I examined patterns of central gustatory activity (via fos-immunohistochemistry) in several central taste structures (nucleus of the solitary tract, parabrachial nucleus, amygdala, and gustatory cortex) following gustatory nerve transection and regeneration. I have also spent years examining how ibotenic lesions of the gustatory cortex affect taste behaviors (taste reactivity, two bottle preference tests).

B. Positions and Honors

Professional Positions

1994-1996 Adjunct Professor of Psychology, Stetson University & Daytona Beach Community College
1996-1998 Visiting Assistant Professor of Psychology/Postdoc, University of Florida
1998-1999 Research Assistant Professor, Stetson University
1999-2000 Lecturer in Psychology, Stetson University
2000- 2001 Visiting Assistant Professor, Psychology Department, Stetson University
2001-2007 Assistant Professor, Psychology Department, Stetson University
2007-2012 Associate Professor, Psychology Department, Stetson University
2012-present Professor, Psychology Department, Stetson University
2016-present Chair, Psychology Department, Stetson University

Honors

1988 First recipient of the Becky Boone Award for Teaching Excellence, University of Virginia
2004 Recipient of the Hand Award for Excellence in Research, Stetson University
2011 Recipient, *William Hugh McEniry Award for Teaching Excellence*, Stetson University
2012 Invited keynote address speaker, 2nd Annual Florida Undergraduate Research Conference

Other Professional Activities

1988-present Associate for Chemoreception Sciences Member

2002-2003	Educational Outreach Program, Association for Chemoreception Sciences
2004-present	Society for the Study of Ingestive Behaviors Member
2004-present	Served as reviewer for the following: *Brain Research, Journal of Comparative Neurology, Chemical Senses, Psi Chi Journal of Undergraduate Research, NCUR Conference Proceeding Papers*, a National Science Foundation Grant, Textbook Chapters for Houghton/Mifflin: Introductory Psychology, Biological Psychology; for Cengage: Introductory Psychology; for Oxford University Press: Behavioral Neuroscience
2010 & 2012	Session Presider, International Conference for Learning and the Brain Conference
2011	Association for Chemoreception Sciences, Election Committee Member
2016-present	Association for Heads of Departments of Psychology (AHDP) Member

C. Contributions to Science

1. My dissertation work revealed for the first time how the developing central gustatory system is altered as a consequence of maternal sodium chloride deprivation.

   King CT and Hill DL (1991). Dietary sodium chloride deprivation throughout development selectively influences the terminal field organization of gustatory afferent fibers projecting to the rat nucleus of the solitary tract. *Journal of Comparative Neurology*, *303*:159-169. https://doi.org/10.1002/cne.903030114

   King CT and Hill DL (1993). Neuroanatomical alterations in the rat nucleus of the solitary tract following early maternal NaCl deprivation and subsequent NaCl repletion. *Journal of Comparative Neurology*, *333*:531-42. 10.1002/cne.903330406

2. My postdoctoral work and continued collaborations with Dr. Alan Spector while I have been at Stetson demonstrated the regenerative capacity of the central gustatory system. (* denotes undergraduate)

   King CT, Travers SP, Rowland NE, Garcia M, Spector AC (1999). Glossopharyngeal nerve transection eliminates quinine-stimulated fos-like immunoreactivity in the nucleus of the solitary tract: Implications for a functional topography of gustatory nerve input in rats. *Journal of Neuroscience, 19*:3107-3121. https://doi.org/10.1523/JNEUROSCI.19-08-03107

   King CT, Garcea M and Spector AC (2000). Glossopharyngeal nerve regeneration is essential for the complete recovery of quinine-stimualted oromotor rejection behaviors and central patterns of neuronal activity in the nucleus of the solitary tract in the rat. *Journal of Neuroscience, 20*:8426-8434. https://doi.org/10.1523/JNEUROSCI.20-22-08426

   King CT, *Deyrup LD, *Dodson SE, *Galvin KE, Garcea M, and Spector AC (2003). Effects of gustatory nerve transection on quinine-stimulated Fos-like immunoreactivity in the parabrachial nucleus of the rat. *Journal of Comparative Neurology, 465*:296-308. https://doi.org/10.1002/cne.10851

   King CT, Garcea M, and Spector AC (2014). Restoration of quinine-stimulated Fos-immunoreactive neurons in the central nucleus of the amygdala and gustatory cortex following reinnervation or cross-reinnervation of the lingual taste nerves in rats. *Journal of Comparative Neurology, 522*:2498-2517. DOI 10.1002/cne.23546

3. My most recent work has focused on the role of the gustatory cortex in taste behaviors

   King CT, Hashimoto, Garcia M, and Spector AC. (2015). Unconditioned oral-motor taste reactivity elicited by sucrose and quinine is unaffected by extensive bilateral damage to the gustatory zone of the insular cortex in rats. *Brain Research, 1599*: 9-19. DOI: 10.1016/j.brainres.2014.12.035

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

Completed Research Support (the last federally funded project):

2010-2016, NIH R01DC009821, *The Functional Organization of the Central Gustatory System,*

PI (primary grant): Alan C. Spector, Florida State University
Co-I (Subcontract #R01335): Camille Tessitore King, Stetson University
Total Funding (FSU subcontract with Stetson):

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: Michael S. King

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

POSITION TITLE: Professor and Chair of Biology

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
Princeton University, Princeton, NJ	B.A.	06/1986	Biology
University of Virginia, Charlottesville, VA	Ph.D.	01/1992	Neuroscience
University of Michigan, Ann Arbor, MI	Postdoc	07/1993	Central Taste Physiology

A. Personal Statement

Beginning with my post-doctoral training, my research has focused on the neuroanatomy and physiology of the central gustatory system as well as behavioral responses to taste input in rodents. Specifically, my work has examined several aspects of the taste system including the physiology of ganglion and nucleus of solitary tract (NST) neurons, details of the connectivity and neurotransmitters expressed by NST neurons that project to the parabrachial nucleus (PBN), and the role of CNS structures in the behavioral responses to taste input. Recently, I have been combining behavioral and neuroanatomical approaches to determine how the CNS controls taste reactivity responses to gustatory input. In this recent work, Fos-immunochemistry has been the main method by which I have studied role of central gustatory and related structures. I feel that this background will allow me to contribute to Dr. McCluskey's work if needed.

B. Positions and Honors

Professional Positions

1993-1999 Assistant Professor of Biology, Stetson University
1999-2005 Associate Professor of Biology, Stetson University
2005-present Professor of Biology, Stetson University
2001-2006 Chair, Dept. of Biology, Stetson University
2021-present Chair, Dept. of Biology, Stetson University

Honors

1998 Hand Faculty Research Award, Stetson University
2008 McEniry Award for Teaching Excellence, Stetson University

Other Professional Activities

1990-present Society for Neuroscience member
1992-present Association for Chemoreception Sciences member
1995-present National Association of Advisors for the Health Professions member
1993-1998 Council on Undergraduate Research member
2010-2016 Council on Undergraduate Research member
2000 Project Kaleidoscope member

1995-present Reviewer of ~25 manuscripts for scientific journals including: *American Journal of Physiology, Brain Research, Brain Structure and Function, Chemical Senses, Journal of Autonomic Neuroscience, Journal of Neurophysiology, Neuroscience, Neuroscience Letters, Physiology and Behavior, Plos One*

1995-present Reviewed text book chapters for the Raven and Johnson (General Biology), Hadley (Endocrinology), Nordell and Valone (Animal Behavior)

C. Contributions to Science

1. My post-doctoral work and subsequent sabbatical research revealed some details about the physiological properties of central taste neurons. The following publications are reports of this work.

King, M.S., Wang, L. and Bradley, R.M., 1993, Substance P excites neurons in the gustatory zone of the rat nucleus tractus solitarius. *Brain Research 619*:120-130. https://www.ncbi.nlm.nih.gov/pubmed/7690670

King, M.S. and Bradley, R.M., 1994, Relationship between structure and function of neurons in the rat rostral nucleus tractus solitarii. *J. Comparative Neurology 344*:50-64. https://www.ncbi.nlm.nih.gov/pubmed/8063955

Bradley, R.M., King, M.S., Wang, L. and Shu, X., 1996, Neurotransmitter and neuromodulatory activity in the gustatory zone of the nucleus tractus solitarius. *Chemical Senses 21*:377-385. https://www.ncbi.nlm.nih.gov/pubmed/8670717

King, M.S. and Bradley, R.M., 2000, Biophysical properties and responses to glutamate receptor agonists of identified subpopulations of rat geniculate ganglion neurons, *Brain Research 866*: 237-246. https://www.ncbi.nlm.nih.gov/pubmed/10825499

2. My initial work in my current position at Stetson University relied on fluorescent tract tracing and immunohistochemistry to investigate details of the connectivity and neurotransmitter expression of brainstem gustatory neurons. This work lead to the following publications.

Williams, J.B., Murphy, D.M., Reynolds, K.E., Welch, S.J. and King, M.S., 1996, Demonstration of a bilateral projection from the rostral nucleus of the solitary tract to the medial parabrachial nucleus in rat. *Brain Research 737*:231-237. https://www.ncbi.nlm.nih.gov/pubmed/8930370

Gill, C.F., Madden, J.M., Roberts, B.P., Evans, L.D. and King, M.S., 1999, A subpopulation of neurons in the rat rostral nucleus of the solitary tract that project to the parabrachial nucleus express glutamate-like immunoreactivity, *Brain Research 821*:251-262. https://www.ncbi.nlm.nih.gov/pubmed/10064811

King, M.S., 2003, Distribution of immunoreactive GABA and glutamate receptors in the gustatory portion of the nucleus of the solitary tract in rat, *Brain Research Bulletin 60*:241-254. https://www.ncbi.nlm.nih.gov/pubmed/12754086

Harrison, T.A., Hoover, D.B. and King, M.S., 2004, Distinct regional distributions of NK1 and NK3 neurokinin receptor immunoreactivity in rat brainstem gustatory centers, *Brain Research Bulletin 63*:7-17. https://www.ncbi.nlm.nih.gov/pubmed/15121234

3. Over the past twenty years or so my work has explored the role of central gustatory structures in behavioral responses to taste input. These studies required a combination of behavioral and anatomical techniques and led to the following publications.

Galvin, K.E., King, C.T. and King, M.S., 2004, Stimulation of specific parabrachial subnuclei elicits ingestive oromotor behaviors in conscious rats, *Behavioral Neuroscience* 118:163-172. https://www.ncbi.nlm.nih.gov/pubmed/14979793

Morganti, J.M., Odegard, A.K. and King, M.S., 2007, The number and location of Fos-like immunoreactive neurons in the central gustatory system following electrical stimulation of the parabrachial nucleus in conscious rats. *Chemical Senses* 32:543-555. https://www.ncbi.nlm.nih.gov/pubmed/17488743

Riley, C.A. and King, M.S., 2013, Differential effects of electrical stimulation of the central amygdala and lateral hypothalamus on Fos-immunoreactive neurons in the gustatory brainstem and taste reactivity behaviors in conscious rats. *Chemical Senses* 38: 705-717. https://www.ncbi.nlm.nih.gov/pubmed/23978688

King, M.S., 2018, Distribution of Fos-immunoreactive neurons in the gustatory cortex elicited by intra-oral infusion of taste solutions in conscious rats. *Brain Research* 1683:67-77. https://www.ncbi.nlm.nih.gov/pubmed/29371098

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

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: Xin-Yun Lu

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

POSITION TITLE: Professor and Chair

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
Washington State University, Pullman, WA	Ph.D.	06/1998	Pharmacology
University of Michigan, Ann Arbor, MI	Postdoc.	1998-2001	Neuroscience

A. Personal Statement

The long-term goals of my research are to understand the molecular, cellular and circuit bases of neuropsychiatric and neurodegenerative disorders, especially for those with comorbid metabolic disorders. Type 2 diabetes and other metabolic consequences of obesity are associated with higher rates of depression, anxiety and dementia. We have made significant contributions to our understanding of the roles of the adipose-brain axis in the regulation of mood, emotion and cognition. Adipose tissue, the primary site of energy storage, is now recognized as a highly active metabolic and endocrine organ, producing a number of secretory bioactive substances termed adipokines. Based upon our studies on two major adipokines (leptin and adiponectin), we have developed a novel theory of dysfunctional adipose-brain axis in the pathogenesis and treatment of depression, post-traumatic stress disorder (PTSD) and Alzheimer's disease. We are particularly interested in how adipokines interact with specific neural circuits orchestrating behavioral and cognitive responses to emotionally and motivationally relevant stimuli, and how they modulate neuroplasticity and tune adaption to experiences and environments in a sex-specific and age-dependent manner.

a. Yu T, Guo M, Garza JC, Rendon S, Sun XL, Zhang W, **Lu XY** (2011). Cognitive and neural correlates of depression-like behavior in socially defeated mice: An animal model of depression with cognitive dysfunction. Int J Neuropsychopharmacol, 14:303-317. PMCID: PMC3432579
b. Guo M, Lu Y, Garza JC, Li Y, Chua SC, Zhang W, Lu B, **Lu XY** (2012). Forebrain glutamatergic neurons mediate leptin action on depression-like behaviors and synaptic depression. Translational Psychiatry, 2: e83. PMCID: PMC3298113
c. Zhang D, Wang X, Wang B, Garza JC, Fang X, Wang J, Scherer, PE, Brenner R, **Lu XY** (2017). Adiponectin regulates contextual fear extinction and intrinsic excitability of dentate gyrus neurons through AdipoR2 receptors. Molecular Psychiatry, 22(7):1044-1055. PMCID: PMC5491689
d. Kim NS, Wen Z, Liu J, Zhou Y, Guo Z, Xu C, Lin YT, Yoon KJ, Park J, Cho M, Kim M, Wang X, Yu H, Salamuru S, Christian KM, Hsu KS, Xia M, Li W, Ross CA, Margolis RL, **Lu XY**[#], Song H[#], Ming GL[#](2021). Pharmacological rescue in patient iPSC and mouse models with a rare DISC1 mutation. Nature Commun. 2021 Mar 3;12(1):1398. doi: 10.1038/s41467-021-21713-3. ([#]corresponding author)

B. Positions and Honors

Positions and Employment

2001-2003 Research Investigator, Mental Health Research Institute, University of Michigan Medical School

2003-2009 Assistant Professor, Department of Pharmacology, University of Texas Health Science Center
2009-2013 Associate Professor, Department of Pharmacology, University of Texas Health Science Center
2010-2017 Cross-appointed Faculty of Psychiatry, Department of Psychiatry, University of Texas Health Science Center
2013-2017 Professor, Department of Pharmacology, University of Texas Health Science Center
2017- Professor, Department of Neuroscience and Regenerative Medicine, Medical College of Georgia at Augusta University
2019- Chair, Department of Neuroscience and Regenerative Medicine, Medical College of Georgia at Augusta University

Honors, Awards, and Other Professional Activities

2005 Scientist Development Award, American Heart Association
2007 Presidential Distinguished Junior Research Scholar Award, University of Texas Health Science Center
2009- *Ad hoc* reviewer of NIH study sections
2010 Independent Investigator Award, National Alliance for Research on Schizophrenia and Depression
2016-2020 Standing member, NIH/Neuroendocrinology, Neuroimmunology, Rhythms and Sleep Study Section
2020-2022 Standing member, NIH/Behavioral Neuroendocrinology, Neuroimmunology, Rhythms, and Sleep (BNRS)
2016- Member, American College of Neuropharmacology (ACNP)
2017- Georgia Research Alliance Eminent Scholar Chair in Translational Neuroscience
2018-2019 Member, External Scientific Advisory Board for the State University of New York Upstate Medical University

C. Contribution to Science

1. *Glutamatergic neurons and mood-related behaviors.* Leptin and its receptor LepRb are well known to play an important role in the pathogenesis of obesity. We identified novel functions of leptin in regulating different aspects of emotion-related behaviors. We found that leptin produces antidepressant-like effects through activating LepRb in forebrain glutamatergic neurons by reducing stress-evoked glutamate release/transmission at dentate gyrus (DG)-CA3 synapses. Ablation of LepRb in forebrain glutamatergic neurons causes pro-depressive behavior and abolishes leptin-s antidepressant-like effects. Moreover, we found that leptin treatment in intact female mice induces behavioral effects and stimulates hippocampal Akt phosphorylation in the proestrus phase but not in the diestrus phase of the estrous cycle. These effects in female mice can be abolished by ovariectomy and restored by pretreatment with 17β-estradiol in ovariectomized female mice. Our results suggest that leptin regulates mood-related behaviors in a sex-specific and estrous cycle-dependent manner.

a. Guo M, Lu Y, Garza JC, Li Y, Chua SC, Zhang W, Lu B, **Lu XY** (2012). Forebrain glutamatergic neurons mediate leptin action on depression-like behaviors and synaptic depression. Translational Psychiatry, 2: e83. PMCID: PMC3298113
b. Wang X, Zhang D, **Lu XY** (2015). Dentate gyrus-CA3 glutamate release/NMDA transmission mediates behavioral despair and antidepressant-like responses to leptin. Molecular Psychiatry, 20(4):509-19. PMCID: PMC4362753
c. Lei Y, Wang J, Wang D, Li C, Liu B, Fang X, You J, Guo M, **Lu XY** (2020). SIRT1 in forebrain excitatory glutamatergic neurons exerts sexually dimorphic effects on depression-related behaviors and modulates neuronal excitability and synaptic transmission in the medial prefrontal cortex. Molecular Psychiatry, 25:1094–1111.
d. Li C, Meng F, Lei Y, Liu J, Liu J, Zhang J, Liu F, Liu C, Guo M, **Lu XY** (2020). Leptin regulates exon-specific transcription of the Bdnf gene via epigenetic modifications mediated by an AKT/p300 HAT cascade. Molecular Psychiatry. 2020 Oct 26. doi: 10.1038/s41380-020-00922-0. Online ahead of print.

2. Leptin, dopamine neurons and emotional behaviors. Obesity has been associated with anxiety disorders. Current antidepressants are effective for the treatment of anxiety disorders after chronic administration. However, they can worsen anxiety in the initial phase of treatment. We found that both fluoxetine and leptin show antidepressant-like behavioral effects in rodent models, but they exhibit opposite effects on anxiety behavior. Acute administration of leptin produces anxiolytic effects, whereas acute fluoxetine elicits anxiogenic effects. Furthermore, we identified that dopamine neurons in the ventral tegmental area (VTA) mediate leptin's effects on anxiety through the Jak2/Stat3 signaling pathway.

a. Liu J, Garza, JC, Bronner J, Kim CS, Zhang W, **Lu XY** (2010) Acute administration of leptin produces anxiolytic-like effects: A comparison with fluoxetine. Psychopharmacology, 207:535–545. PMCID: PMC4057895
b. Liu J, Perez S, Zhang W, Lodge D, **Lu XY** (2011). Selective deletion of the leptin receptor in dopamine neurons produces anxiogenic-like behavior and increases dopaminergic activity in amygdala. Molecular Psychiatry, 16(10):1024-1038. PMCID: PMC3432580
c. Liu J, Guo M, **Lu XY** (2015) Leptin/LepRb in the ventral tegmental area mediates anxiety-related behaviors. The International Journal of Neuropsychopharmacology, 19(2). pii: pyv115. PMCID: PMC4772826
d. Carrier N, Wang X, Sun L, **Lu XY** (2015) Sex-specific and estrous cycle-dependent antidepressant-like effects and hippocampal Akt signaling of leptin. Endocrinology, 156(10):3695-3705. PMCID: PMC4588814

3. Adiponectin, emotion-related behaviors and fear memories. Adiponectin is a metabolic hormone that is secreted exclusively by adipocytes and can cross the blood-brain barrier to exert effects on the central nervous system beyond metabolic control. We have made major contributions to general understanding how adiponectin mediates the crosstalk between adipocytes and neurons and how adiponectin expression in adipose tissue is regulated by stress and how adiponectin in the brain modulates the negative feedback action of glucocorticoid hormones, neuronal excitability, emotional behaviors and fear memories. We found that adipose PPARγ and adiponectin expression levels are downregulated after exposure to chronic stress and correlate with changes in behavioral responses. Central infusion of adiponectin induces antidepressant and anxiolytic effects. By contrast, adiponectin insufficiency increases susceptibility for stress-induced depressive- and anxiety-like behaviors. Moreover, we found that adiponectin in the ventral tegmental area inhibits dopamine neuron (DA) population activity and can reverse stress-induced increase in DA neuron firing and loss of AdipoR1 specically in DA neurons induces anxiogenic behavior and enhances DA neuron responses to stress. In the hippocampus, adiponectin suppresses neuronal excitability of hippocampal granule neurons and modulates extinction of contextual fear memories in a mouse model of post-traumatic stress disorder. Adiponectin deficiency impairs fear extinction, whereas intra-hippocampal infusions of adiponectin facilitate extinction of contextual fear.

a. Liu J, Guo M, Zhang D, Cheng SY, Liu M, Ding J, Scherer PE, Liu F, **Lu XY** (2012). Adiponectin is critical in determining susceptibility to depressive behaviors and has antidepressant-like activity. Proc Natl Acad Sci U S A, 109(30):12248-12253. PMCID: PMC3409774
b. Guo M, Li C, Lei Y, Xu S, Zhao D, **Lu XY** (2017). Role of the adipose PPARγ/adiponectin axis in susceptibility to stress and emotion-related disorders. Molecular Psychiatry, 22(7):1056-1068. PMCID: PMC5468488
c. Zhang D, Wang X, Wang B, Garza JC, Fang X, Wang J, Scherer, PE, Brenner R, **Lu XY** (2017). Adiponectin regulates contextual fear extinction and intrinsic excitability of dentate gyrus neurons through AdipoR2 receptors. Molecular Psychiatry, 22(7):1044-1055. PMCID: PMC5491689
d. Sun F, Lei Y, You J, Li C, Sun L, Garza J, Zhang D, Guo M, Scherer P, Lodge D, **Lu XY** (2019). Adiponectin modulates ventral tegmental area dopamine neuron activity and anxiety-related behavior through AdipoR1. Molecular Psychiatry, 24:126–144. PMID: 29988086

2. Adipokines, neurogenesis and neuroplasticity. Remodeling of dendritic spines and synapses of existing neurons and adult neurogenesis in the hippocampus are critical for memory and emotional processing. Using in

vitro and in vivo models, we found that leptin and adiponectin, two major adipokines, promote adult neurogenesis in the hippocampus via different intracellular signaling mechanisms. Moreover, we observed that adiponectin elicits neurotrophic effects on dendritic growth, arborization and spinogenesis. Our studies indicate that adipokines modulate two forms of structural plasticity in the hippocampus, i.e. morphological modifications of dendritic spines/synapses of existing neurons and generation of new neurons in adulthood.

a. Garza JC, Guo M, Zhang W, **Lu XY** (2008). Leptin increases adult hippocampal neurogenesis in vivo and in vitro. The Journal of Biological Chemistry, 283(26):18238-47. PMCID: PMC2440628
b. Zhang D, Guo M, Zhang W, **Lu XY** (2011). Adiponectin stimulates proliferation of adult hippocampal neural stem/progenitor cells through activation of p38MAPK/GSK-3β/β-catenin signaling cascade. The Journal of Biological Chemistry, 286(52):44913-20. PMCID: PMC3247954
c. Garza JC, Guo M, Zhang W, **Lu XY** (2012). Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3β/β-catenin signaling. Molecular Psychiatry, 17(8):790-80. PMCID: PMC3368076
d. Zhang D, Wang X, **Lu XY** (2016). Adiponectin exerts neurotrophic effects on dendritic arborization, spinogenesis and neurogenesis of the dentate gyrus of male mice. Endocrinology, 157(7):2853-2869. PMCID: PMC4929553

5. Central melanocortin signaling, HPA and stress responses. The central melanocortin system has been extensively investigated for its role in the control of feeding behavior and body weight. Our anatomical studies show that POMC and AgRP neurons, the two major components of the melanocortin system, interact with the stress system. We found that corticotrophin releasing hormone (CRH) neurons in the paraventricular nucleus relay melanocortin signaling to influence the hypothalamo-pituitry-adrenal (HPA) axis activity and food intake. We demonstrated that melanocortins induces stress-like responses, such as anorexia, anxiety and HPA activation. Using emotional stressors, we compared the responsiveness of POMC and AgRP neurons and discovered that these neurons in the arcuate nucleus are rapidly but differentially recruited by emotional stress. This body of work emphasizes the important role of melanocortinergic signaling in mediating endocrine, emotional and feeding responses to acute and chronic stress.

a. Liu J, Garza JC, Truong VTH, Henschel J, Zhang W, **Lu XY** (2007). The melanocortinergic pathway is rapidly recruited by acute emotional stress and contributes to stress-induced anorexia and anxiety-like behavior. Endocrinology, 148(11): 5531-5540. PMCID: PMC3708592
b. Liu J, Garza JC, Li W, **Lu XY** (2013). Melanocortin-4 receptor in the medial amygdala regulates emotional stress-induced anxiety-like behaviour, anorexia and corticosterone secretion. The International Journal of Neuropsychopharmacology, 16(1):105-120. PMCID: PMC3708461
c. Fang X, Jiang S, Wang J, Bai Y, Kim CS, Blake D, Wentraub NL, Lei Y, **Lu XY** (2021). Chronic unpredictable stress induces depression-related behaviors by suppressing AgRP neuron activity. Molecular Psychiatry, 2021 Jan 11;10.1038/s41380-020-01004-x. doi: 10.1038/s41380-020-01004-x. Online ahead of print.
d. Fang X, Chen Y, Wang J, Zhang Z, Bai Y, Denney K, Gan L, Guo M, Weintraub NL, Lei Y, **Lu XY**. Activation of hypothalamic POMC neurons is sufficient and necessary to induce depression-related behaviors. Molecular Psychiatry. Under revision.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1rAyIBH9C4ikN/bibliography/public/

D. Research Support

1 RF1 AG062166-01 (Lu) 02/15/2019 – 01/31/2024
NIH/NIA
"The adipocyte PPARgama/adiponectin axis and Alzheimer's disease"
Major Goal(s): To examine the role of the adipocyte-brain PPARγ/adiponectin/AdipoR axis in the onset and progression of Alzheimer's disease.

Role: Principal Investigator

1R56 MH119456-01 (Lu) 04/01/2019 – 02/28/2022 (NCE)
NIH/NIMH
"Distinct roles of POMC and AgRP neurons in chronic stress-induced depressive behaviors"
Major Goal(s): To study how hypothalamic POMC and AgRP neurons modulate chronic stress-induced depressive behaviors.
Role: Principal Investigator

1R56AG064895-01 (Lu/Weintraub) 09/15/2019 – 06/30/2022 (NCE)
NIH/NIA
"HDAC9, Aging and Alzheimer's Disease"
Major Goal(s): This application aims at identifying the molecular bases underlying the effects of aging on AD-related neuropathology and cognitive deficits. We expect that the results will provide insights into therapeutic interventions targeting specific subtypes of HDACs and tissue-selective therapy.
Role: Co-Principal Investigator

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

2. *Program Income Section

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

○ Yes ● No

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

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

Tracking Number: GRANT13439458

Funding Opportunity Number: PA-20-195 . Received Date: 2021-08-05T18:21:52.000-04:00

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

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

If "yes" then provide the HFT Compliance Assurance

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

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

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

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT13439458 Funding Opportunity Number: PA-20-195 . Received Date: 2021-08-05T18:21:52.000-04:00

PHS 398 Modular Budget

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

Budget Period: 1

Start Date: 04/01/2022 End Date: 03/31/2023

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	[redacted]
Consortium Indirect (F&A)	
Total Direct Costs*	[redacted]

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Total Modified Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 11/24/2020

Total Indirect (F&A) Costs: [redacted]

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): [redacted]

Tracking Number: GRANT13439458
Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 04/01/2023 End Date: 03/31/2024

A. Direct Costs

Funds Requested ($)

Direct Cost less Consortium Indirect (F&A)* [redacted]

Consortium Indirect (F&A)

Total Direct Costs* [redacted]

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Total Modified Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, Arif Karim, (214) 767-3261

Indirect (F&A) Rate Agreement Date 11/24/2020

Total Indirect (F&A) Costs [redacted]

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($) [redacted]

Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	[redacted]
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	[redacted]
Section A, Total Direct Costs for Entire Project Period ($)	[redacted]
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	[redacted]
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	[redacted]

2. Budget Justifications

Personnel Justification	McCluskey_Personnel_Just2.pdf
Consortium Justification	
Additional Narrative Justification	

Tracking Number: GRANT13439458 Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

PERSONNEL JUSTIFICATION

Name	Role	Person Months on Project
Lynnette McCluskey, Ph.D.	PI	2.04
Lin Gan, Ph.D.	PI	0.96
Daniel Linder, Ph.D.	Collaborator	0.3
Camille Tessitore King, PhD	Consultant	0
Michael King, PhD	Consultant	0
Xin-Yun Lu, MD/PhD	Consultant	0
Guangkuo Dong, MS	Research Associate	6.0
Schuyler Kogan, BS	Research Assistant	10.2

Lynnette McCluskey (PI) is a project PI. She will oversee and be responsible for all experiments. Dr. McCluskey will analyze results, perform imaging experiments, and write and edit manuscripts detailing results.

Lin Gan (PI) is a project PI. He will oversee and be responsible for all mouse breeding and genotyping. Dr. Gan will analyze results, perform experiments, and edit manuscripts detailing results.

Daniel Linder (Co-I) will perform mixed-model statistical analyses for the project.

Camille Tessitore King (Consultant) will provide guidance on mapping Ace2+ cells in central taste structures in Aim 1.

Michael King (Consultant) will provide guidance on mapping Ace2+ cells in central taste structures in Aim 1. We anticipate that undergraduate students in his laboratory will analyze images uploaded to a shared account.

Xin-Yun Lu (Consultant) will advise on brain regions outside the taste system that may contain novel populations of *Ace2*+ cells. We will first focus on regions near taste structures using conventional anatomical techniques but expect that laboratory personnel will be trained on a new light-sheet microscope by early 2022.

Guangkuo Dong (Research Associate) will perform experiments (qPCR, ELISAs, and neurophysiology). He is highly skilled at recording chorda tympani responses in mice.

Schuyler Kogan (Research Assistant) will perform immunoassays, confocal imaging and image analyses for Aims 1 and 2.

PHS 398 Research Plan

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

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	McCluskey_Introduction_FINAL.pdf
Research Plan Section	
2. Specific Aims	McCluskey_Aims2.pdf
3. Research Strategy*	McCluskey_Research_FINAL.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	McCluskey_Animals2.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	McCluskey_LeadershipPlan.pdf
8. Consortium/Contractual Arrangements	
9. Letters of Support	Compiled_Letters.pdf
10. Resource Sharing Plan(s)	RESOURCE_SHARING_PLAN2.pdf
11. Authentication of Key Biological and/or Chemical Resources	McCluskey_Authentication2.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT13439458
Funding Opportunity Number: PA-20-195. Received Date: 2021-08-05T18:21:52.000-04:00

INTRODUCTION

We thank the reviewers for their helpful comments on our previous R21 submission. The reviewers agreed that the proposed studies to investigate the role of ACE2 in the taste system of novel mouse models are "highly significant" given our meager understanding of taste loss due to SARS-CoV-2 infection. Reviewers also noted that the "PI, her colleagues, and the research environment were judged outstanding, fully capable of bringing the project to fruition as designed". We respond to reviewers' comments and summarize revisions to the current application below.

ACE-2 expression in taste buds.

- Two reviewers commented that ACE2 staining in taste buds may be non-specific. We now include negative control images for ACE2 (Fig. 1) and TMPRSS (Fig. 2) immunofluorescence demonstrating antibody specificity.

- When the previous application was submitted the premise that taste cells express ACE2 was more controversial. *Ace2* expression was reported in a just a small subset of mouse type III taste cells by RNA-seq (Wang et al., 2020) although preliminary transcriptional profiling of public databases indicated more widespread expression in subsets of type II and III taste cells (Cooper et al., 2020). *Ace2* expression in mouse taste buds was also demonstrated by PCR though only mentioned briefly since *Ace1* was the focus of that study (see Fig. 1A by Shigemura et al., 2019). In our preliminary results, ACE2 immunoreactivity was widely detected in anterior and posterior mouse taste buds. Since the previous submission, ACE2 expression in type II human taste cells was shown by immunostaining and RNAscope *in situ* hybridization (Doyle et al., 2021). In Aim 1 we propose to use a novel reporter mouse strain to map *Ace2* expression with high sensitivity, resolving discrepancies between detection methods.

Transgenic approaches

- One reviewer was positive about mapping Ace2 expression in the taste system using the proposed reporter mice. Another reviewer commented that co-staining with cellular markers will be overly complicated in *Ace2* knock-in mice expressing both TdTomato and GFP. Therefore we have revised our design to replace GFP with the V5 epitope. We have recently generated Ace2-P2A-V5-CreERT2 (*Ace2*^{CE}) knock-in founder mice using CRISPR/Cas9 method (Fig. 4) and will breed this new *Ace2*^{CE} line with Ai14 Rosa26-tdTomato reporter mice. This strategy allows us to trace the lineage of *Ace2*+ cells with high sensitivity and straightforward immunolabeling with cell-specific markers.

- The keratin (K14) promoter allows lingual epithelium-specific but not taste cell-specific *Ace2* knockout. This approach is widely used in the field but has implications for interpreting results as now addressed (Aim 2a).

- As suggested we have added collaborators with anatomical expertise in central taste regions (Dr. Michael King and Dr. Camille Tessitore King) and brain regions outside taste structures (Dr. Xin-Yun Lu). The Dept. of Neuroscience & Regenerative Medicine has also added a new workstation with NeuroInfo software which outlines brain regions on images using the Allen Mouse Brain Atlas. Thus we have the mouse models, equipment and collaborative expertise to map *Ace2* expression in the central and peripheral taste system for Aim 1.

SPECIFIC AIMS

The virally-mediated disease known as COVID-19 has infected and killed millions worldwide. COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is characterized by the loss of taste, smell and chemesthesis sometimes in the absence of other common symptoms such as respiratory distress, fatigue, cough, and fever. Taste deficits are prevalent and appear to be independent from anosmia. However, the biological basis of taste loss is largely unknown, including the potential for SARS-CoV-2 infection and ensuing inflammation in taste receptor cells and ascending neural pathways. A fundamental question is whether central and peripheral taste structures are targets of SARS-Cov-2. Insight to host viral entry pathways and their normal function in taste buds is needed before SARS-Cov-2 induced taste deficits can be treated. While most patients regain taste sensation after several weeks, hypoguesia is longer lasting in a subset of patients leading to prolonged negative effects on nutrition and quality of life.

The angiotensin-converting enzyme (ACE) 2 receptor is a negative regulator of the renin-angiotensin system (RAS) that mediates fluid balance and blood pressure. RAS hormones angiotensin II and aldosterone also modulate taste function, behavior, and immune responses to taste nerve injury. A member of the RAS, ACE2, binds the SARS-CoV-2 spike protein. Prior to host cell entry the spike protein must be cleaved by proteolytic enzymes such as furin, cathepsins, and most prominently, TMPRSS2. ACE2 is downregulated as receptor bound virus is internalized by host cells, driving the RAS to proinflammatory, vasoconstrictive, and fibrotic actions that contribute to organ damage. Thus ACE2 is essential for SARS-CoV-2 infection but also mediates protective responses through the RAS. *Our preliminary results demonstrate robust ACE2 and TMPRSS2 expression in anterior and posterior mouse taste buds.*

Mouse models were highly useful in understanding the related SARS-CoV tropism and pathogenesis responsible for the severe acute respiratory syndrome (SARS) epidemic of 2002-2004. Yet the murine ACE2 receptor binds SARS-CoV-2 ineffectively compared to human ACE2. This R21 Exploratory/Developmental Research application addresses the urgent need to understand the role of ACE2 in the taste system using suitable animal models. *We have developed novel genetic mouse strains to address this gap in knowledge*. We hypothesize that ACE2 is expressed in multiple taste cell types, modulates taste nerve responsivity, and has an anti-inflammatory, protective influence on the peripheral taste system. We also test whether ACE2 bound to SARS-CoV-2 spike protein has detrimental effects on taste receptor cells and taste function which may contribute to taste deficits in COVID-19 patients. We test these hypotheses in two aims:

Aim 1. Identify ACE2-expressing cells in peripheral and central taste pathways as potential targets of SARS-CoV-2. We will map ACE2 expression using standard cell markers in a novel *Ace2-P2A-CreERT2* knock-in *($Ace2^{CE}$)* strain of mice which express inducible CreERT2 at the endogenous *Ace2* locus combined with Rosa26-tdTomato reporter mice. We will use this strain combined with antibodies to identify ACE2+ taste cell types, neurons, glia and blood vessels.

Aim 2. Determine the contribution of lingual ACE2 to taste function and taste receptor cell dynamics in the healthy and inflamed peripheral taste system and potential modulation by SARS-CoV-2 spike protein.

a. We will record responses to taste and tactile stimuli from a primary taste afferent nerve, the chorda tympani (CT), and quantify taste receptor cell number and turnover in lingual epithelium-specific *Ace2* knockout mice using a new floxed *Ace2* strain. Conditional *Ace2* knockout and control mice will also be treated with systemic lipopolysaccharide (LPS) to test taste function during lingual inflammation.

b. We will record neural responses and measure taste cell dynamics in humanized *Ace2* knock in mice (*hAce2-KI)* which recapitulate endogenous ACE2 expression. Mice will be challenged with LPS and/or a human SARS-CoV-2 spike-Fc fusion protein to determine the impact on taste function.

We expect taste changes in the absence of ACE2 reflecting the dysregulation of the RAS. Inflammation (Aim 2a) and/or downregulation of ACE2 by spike-Fc (Aim 2b) are expected to exacerbate taste alterations based on a similar approach in a mouse lung injury model.

These studies address the compelling need to understand ACE2 function in the taste system. If taste buds and associated pathways are indeed potential SARS-CoV-2 targets, we will test the effects of pseudovirus typed with human SARS-CoV-2 spike protein on taste function and behavior in *hACE2-KI* mice in future studies.

RESEARCH STRATEGY

1. Significance

The ongoing global pandemic caused by the novel coronavirus SARS-CoV-2 has resulted in millions of infections and deaths. Smell and taste deficits are major neurological symptoms of COVID-19 occurring in up to 80% of those infected often in the absence of respiratory ailments and fever[1-9]. Chemosensory symptoms are highly predictive of SARS-CoV-2 infection and hospitalizations[10-12]. Chemesthesis, mediated by sensory neurons in the lingual epithelium, also contributes to flavor perception. Recent reports indicate that loss of taste and chemesthesis can occur independently from anosmia[2,3,6]. This is consistent with prior studies demonstrating taste deficits after upper respiratory viral infection and inflammation[13-15]. Most patients regain taste sensation after recovery from viral infection but taste deficits persist in some people[16-19]. Taste is important for nutrition and quality of life lending urgency to the search for mechanisms responsible for hypoguesia in COVID-19 patients[20-25]. ***There is currently little known about the SARS-CoV-2 infection and associated inflammation in the taste system.*** *This R21 application responds to the Notice of Special Interest (NOT-DC-20-008) from NIDCD focused on "the molecular mechanisms underlying chemosensory dysfunction due to SARS-CoV-2 infection".*

Angiotensin-converting enzyme (ACE)2 in the renin-angiotensin system (RAS) and SARS-CoV-2 infection. [26]Coronaviruses are composed of a sphere of structural proteins coated with a "corona" of viral spike protein[27]. The receptor for the SARS-CoV-2 spike protein is ACE2[28,29], a negative regulator of the renin-angiotensin-system (RAS) which controls extracellular fluid volume and blood pressure[30]. In one wing of the RAS, ACE converts Ang I to Ang II which binds the AT1 receptor leading to vascular constriction, fibrosis, oxidative stress, and inflammation (Fig. 1). ACE2 counteracts these effects by converting Ang II to Ang (1-7) inducing vascular dilation, anti-fibrosis and anti-inflammatory effects[30,31]. Thus ACE2 is protective in the context of the RAS. Conversely, ACE2 enables SARS-CoV-2 infection, and the widespread expression of ACE2 throughout tissues is thought to underlie multi-organ damage in patients with severe COVID-19[31,32]. Proteases are also needed to activate the ACE2-spike protein enabling viral fusion to the host cell membrane and internalization[28,29] (Fig. 1). Host transmembrane protease, serine 2 (TMPRSS2) appears to be the most prominent spike activating protease though cathepsins[28], furin[33], and neuropilin-1 (NRP-1) are also co-factors[34]. In Aim 1 we map endogenous *Ace2* expression in the taste system of *Ace2*^{CE} reporter mice to identify potential SARS-CoV-2 infection sites underlying taste deficits.

RAS modulation of taste and inflammation. In the classic RAS feedback loop Ang II stimulates the adrenal cortex to produce aldosterone which causes Na^+ reabsorption via epithelial Na^+ channels (ENaCs)[35]. ENaC also transduces Na^+ in taste buds[36-39]. Acute aldosterone treatment increases ENaC expression[40] and function[40] in taste receptor cells and elevates amiloride-sensitive CT responses to Na^+ [41]. Chronic aldosterone treatment in combination with contralateral CT nerve injury[42] or with Na^+ in drinking water[43] inhibited CT responses to Na^+. Early macrophage responses to CT nerve sectioning were also reduced by aldosterone in contrast to the hormone's well-established proinflammatory role in chronic cardiovascular injury[44]. The tissue microenvironment, injury model, and length of aldosterone treatment may account for different effects on salt taste responsivity and inflammation[44-46]. Ang II, upstream from aldosterone, suppressed amiloride-sensitive CT responses to Na^+ but enhanced sweet responses. The authors suggest that Ang II increases Na^+ ingestion by rapidly decreasing neural Na^+ sensitivity before aldosterone restores homeostasis by elevating amiloride-sensitive Na^+ responses[47]. The Ang II receptor, AT1, is co-expressed with αENaC or the sweet taste receptor subunit, T1r3, in taste receptor cells demonstrating another role of RAS in the peripheral taste system[47]. ACE2's role in taste cell function is unknown[48]. ***We will gain insight to the contribution of ACE2 to peripheral taste function under baseline and inflammatory conditions using lingual epithelial-specific Ace2 (LE-Ace2) knockout mice.***

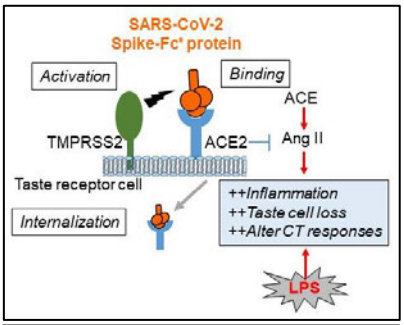

Fig. 1 ACE2 in the peripheral taste system. We propose hat taste cells express ACE2 (**Aim 1**) as a substrate for SARS-CoV-2 spike protein binding and entry. In **Aim 2a** we test whether taste nerve responses and taste cell dynamics are altered in the absence of lingual epithelium-specific *Ace2*, a negative regulator of Angiotensin (Ang) II, which drives the renin-angiotensin system (RAS) to a destructive phenotype in lung. We will test taste function in humanized *Ace2* mice in **Aim 2b**. Lipopolysaccharide (LPS) induced inflammation and SARS-CoV-2 spike-Fc protein are expected to further worsen taste outcomes.

ACE2 and lung injury. ACE2 is protective in mouse models of severe lung injury induced by LPS, sepsis and acid aspiration[49-53]. ACE2 knockout worsened lung function in injury models while exogenous ACE2 acting via

the AT2 receptor protects against lung damage[50]. Mice with acid-induced lung injury were given systemic SARS-CoV spike protein to establish a model that mimics SARS-CoV infection[52]. This model was critical in demonstrating viral tropism and pathogenesis in the lung[49,51,52]. The major limitation in following the same blueprint for the current pandemic is the ineffective binding of SARS-CoV-2 to the murine ACE2 receptor. *We have addressed this problem by generating novel mouse models including humanized Ace2 knock in mice (hAce2-KI) which recapitulate endogenous Ace2 expression*. In an approach used in lung, in Aim 2b we will determine taste effects of delivering a chimeric human spike protein to humanized ACE2 mice in combination with systemic LPS, which stimulates inflammation and alters taste cell dynamics and taste responses[54,55].

Expression of ACE2 and associated SARS-CoV-2 entry molecules in taste buds. ACE2 expression in the human tongue and oral cavity has been reported[56-60] most recently in type II taste cells bearing GPCRs for sweet, bitter and umami stimuli and near taste buds in post-mortem circumvallate papillae and fungiform biopsies[61]. In the latter study, replicating SARS-CoV-2 was detected in type II taste cells in fungiform papillae and taste stem cell proliferation was decreased by viral infection in patient biopsies. This study, while small, suggests that direct infection of type II taste cells could explain deficits in sweet, bitter and umami taste stimuli while indirect mechanisms could impact salt and sour taste perception[61]. ACE2, TMPRSS2 and furin expression in human taste cell cultures have also been reported[56].

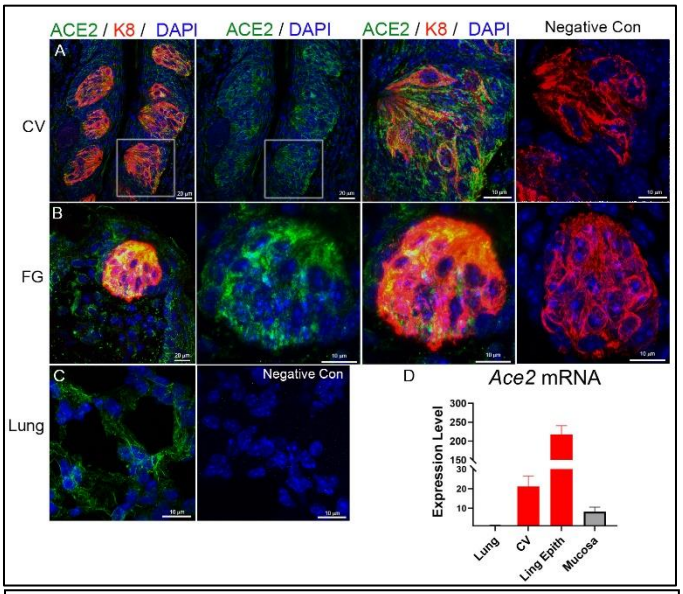

Fig. 2 Robust ACE2 expression in mouse taste buds. ACE2 expression (green) in (**A**) circumvallate (CV) and (**B**) fungiform (FG) taste buds labeled with the taste cell marker keratin (K)8 (red). CV taste buds in white squares are shown at higher magnification on the right. ACE2 antibody was omitted in negative control sections and laser settings matched to within-assay positive tissues. (**C**) Lung positive tissue control and nega ive staining control. Results are representative of n=3-4 mice in assays performed ≥3x. (**D**) *Ace2* mRNA expression in CV papillae, anterior lingual epithelium containing fungiform taste buds and lingual mucosa. Transcript levels are expressed relative to lung which was set as 1 (n=3 mice/tissue).

Whether rodent taste buds express ACE2 is currently less clear. *Ace2* was only detected in a small subset of mouse type III taste cells and lingual epithelial cells using RNA-Seq[62]. Preliminary data mining from public databases indicated more widespread *Ace2* expression in subsets of mouse type II, III and LGR-5+ taste stem cells[63]. A type I cell database was not available[63]. Though a minor part of the publication, *Ace2* was also demonstrated in mouse taste buds and non-taste epithelium[64]. ACE2 and TMPRSS immunoreactivity was reported in rat taste buds though not localized to specific types[65]. Tmprss2 was not detected in type II or III taste cells in other analyses[63].

Preliminary results. We demonstrate robust immunoreactivity for ACE2 (Fig. 2) and TMPRSS2 (Fig. 3) in K8+ taste cells from C57BL/6J mice. ACE2 and TMPRSS2 staining largely overlaps with K8, expressed by mature taste cells[66], indicating widespread expression in anterior and posterior lingual taste buds. We verified our results using lung as a positive control for ACE2 (Fig. 2C) and TMPRSS2 (Fig. 3C)[67,68]. Minimal staining was observed in negative control sections in which primary antibody was omitted (Fig. 2 and 3). Compared to lung, *Ace2* mRNA expression levels are elevated in the CV papillae and anterior lingual epithelium containing fungiform taste buds (Fig. 2D). *Tmprss2* expression levels are similar in lung, CV and anterior lingual epithelium (Fig. 3D). The reason for the difference between preliminary results and RNA-seq studies[62,63] is unclear. In Aim 1 we resolve this discrepancy with a new reporter mouse to identify even weakly-positive and transiently-expressing *Ace2*+ cells.

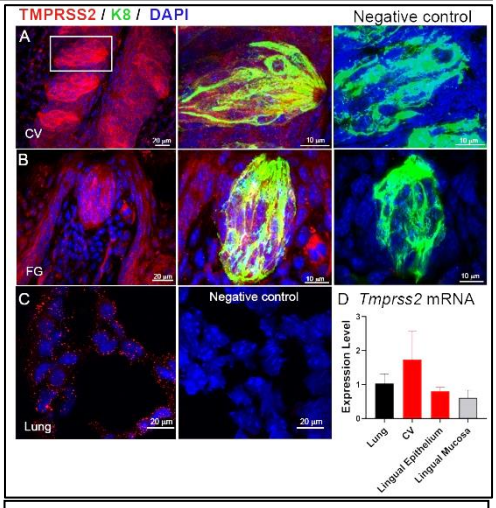

Fig. 3 TMPRSS2 expression in mouse taste buds. TMPRSS2 is expressed in K8+ taste buds in (A) CV and (B) FG papilla andTMPRSS2-omitted negative control. Positive and negative staining is also shown in (C) lung. Results are representative of n=3-4 mice in assays performed 3x. (E) *Tmprss2* mRNA expression levels in the CV and anterior lingual epithelium were similar to those in lung which was set as 1 (n=3 mice / tissue).

To facilitate the identification of *Ace2*-expressing cells and the functional study of ACE2 in the taste tissue, we have been creating in the C57BL/6J background the following three novel *Ace2* mouse models

(Fig. 4 and Table 1): (1) *Ace2-P2A-CreERT2* (*Ace2*^{CE}) knock-in mice: a tamoxifen-inducible CreERT2 (CE) fusion protein is inserted in-frame to the C-terminus of endogenous ACE2, an approach that we have recently used to create *Gfi1*^{GCE} mice[69].

The self-cleaving P2A peptide allows the co-expression of CE from the endogenous *Ace2* locus without disrupting ACE2 expression and function. In Aim 1, we will use this *Ace2*^{CE} strain to activate reporter gene expression in *Ace2*-expressing cells and determine the taste cell types and primary taste structures potentially targeted by SARS-CoV-2. (2) *Ace2* conditional knockout (*Ace2*^{loxP}) mice: Exon 4 is floxed by loxP and can be deleted by Cre recombinase to cause a shift in reading-frame and the inactivation of *Ace2*. This strain allows study of *Ace2* function in tissue/cell type-specific fashion; and (3) Humanized *Ace2* knock-in (h*Ace2*-KI) mice: Human *Ace2* and SV40 polyadenylation sequences are knocked in the first *Ace2* coding exon (exon 2) and in-frame with the translation initiation Met codon at mouse *Ace2* locus. Thus, hACE2 expression is driven by the mouse endogenous *Ace2* promoter and should recapitulate the expression of endogenous ACE2. *Ace2*^{loxP} and *hAce2*-KI mice will be used for neurophysiological studies in Aim 2. Currently, we have successfully generated germline-transmitted h*Ace2*-KI and *Ace2*^{loxP} mice as well as *Ace2*^{CE} (see PCR genotyping confirmation of heterozygous mice in Fig. 4) and expect to obtain *Ace2*^{CE} germline transmitted heterozygous mice by September, 2021.

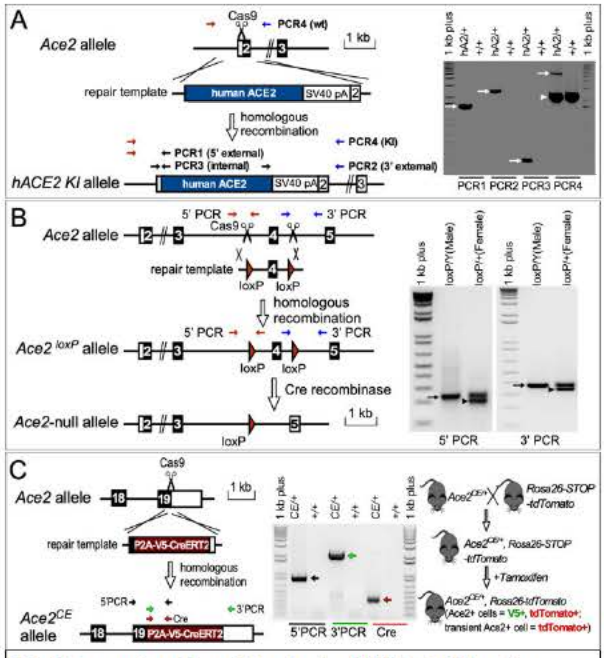

Fig. 4 Generation of novel *Ace2* mice. (A) *hAce2-KI* targeting strategy and PCR genotyping confirmation of *hAce2-KI* heterozygotes. (B) *Ace2*^{loxP} targeting strategy and PCR genotyping confirmation of *Ace2*^{loxP} mice. (C) *Ace2*^{CE} targeting strategy and lineage tracing of *Ace2*-expressing cells by tamoxifen-inducible CE and Rosa26-tdTomato reporter gene. Arrowheads and arrows indicate WT and targeted *Ace2* PCR fragments, respectively.

Table 1: Novel mouse strains used in proposal

Strain	Description	Use
Ace2-P2A-CreERT2 knock-in (*Ace2*^{CE})	Expresses CreERT2 at the endogenous *Ace2* locus combined with the tdTomato from Ai14 Rosa26-STOP-tdTomato reporter mouse line	Aim 1: Map *Ace2* expression in the peripheral and central taste system
Ace2 conditional knockout mice (*Ace2*^{loxP})	Bred to K14-cre mice (JAX #018964) to generate taste receptor cell and lingual epithelium-specific knockout strain *LE-Ace2 KO*	Aim 2a: Role of LE-*Ace2* in taste function, dynamics, and response to LPS challenge
Humanized *Ace2* knock-in mice (*hAce2-KI*)	Expresses he human *Ace2* gene under the control of the endogeous *Ace2* regulatory sequence	Aim 2b: Test taste func ion, dynamics, and response to human spike-Fc protein +/- LPS-induced taste inflamma ion

Scientific premise. ACE2 expression in taste buds is supported by our preliminary results, recent studies using human samples[56-61], and some[64,65] (or partially in agreement with[63]) but not all[62] reports using rodent samples. Experiments proposed in Aim 1 using the novel *Ace2*^{CE} mouse strain will resolve inconsistencies. The premise that SARS-CoV-2 causes taste loss is widely accepted driving the need for biological insight to underlying mechanisms[2,63,70]. Strong evidence confirms SARS-CoV-2 entry mechanisms through ACE2 and TMPRSS similar to SARS-CoV[28,71]. Additional proteases activate SARS-CoV-2 in other systems[72,73], though which mechanisms prevail in taste buds is unknown[63]. Previous studies demonstrate the role of ACE2 in SARS-CoV pathogenesis in the lung[49-51,53]. This work provides a guide for developing a clinically-relevant mouse model to test taste changes caused by the human SARS-CoV-2 spike protein. The involvement of the RAS in taste buds is also supported by evidence from multiple laboratories over time[40-43,47]. Whether ACE2 counteracts Ang II to modulate taste function and inflammatory responses is unknown (Fig. 1), leading to a major gap in our understanding of this fundamental homeostatic system addressed in the proposed studies.

2. Innovation

The rapid emergence and spread of SARS-CoV-2 created a lag between the recognition of taste deficits in COVID-19 patients and insight to biological underpinnings. Major limitations in discovering mechanisms underlying taste loss have included limited mouse models due to ineffective binding between the SARS-CoV-2 spike protein and mouse ACE2[74], the lack of BSL3 facilities needed to work with the virus, and the shortage of investigators trained in both chemosensation and immunology. ***We resolve each limitation in this R21 Exploratory /Developmental application:*** (1) Dr. Gan used CRISPR/Cas9 to develop new mouse models including a humanized *hAce2-KI* strain (Table 1); (2) human spike-Fc protein will be used in *hAce2-KI* mice to define inflammatory responses and taste loss in standard labs; and (3) Dr. McCluskey trained in prominent

taste and neuroimmunology laboratories and has studied the taste-immune axis independently for >20 years. ACE2 in our humanized mice is expressed at the endogenous locus and is physiologically relevant. The use of the K18 promoter to drive ACE2[75,76] expression in commercially-available mice is of particular concern since a subset of rodent[66,77,78] and human[79] taste receptor cells express K18. One *hAce2*-KI strain, newly available from Jackson Laboratory[80], is available if needed for Aim 2b (Alternative outcomes). We have encountered unexpected hair cell and taste bud regeneration phenotypes in transgenic strains highlighting the need for multiple *Ace2* mouse models. Together the novel mouse models and combined expertise of the team will enable fundamental insights to ACE2 and spike protein function in the taste system.

3. Approach.

In Aim 1 we map ACE2 expression in the taste system using novel *Ace2^{CE}* and Rosa26-tdTomato reporter mice. In Aim 2 we use the conditional *LE-Ace2 KO* and *hAce2-KI* to test the role of ACE2 in taste function, inflammation, and in response to human SARS-CoV-2 spike protein.

Scientific rigor, reproducibility, and sex as a biological variable. We determined group sizes by power analyses and chose statistical analyses in collaboration with Dr. Daniel Linder in the Dept. of Biostatistics & Epidemiology at the Medical College of Georgia. Analyses will be performed by personnel blinded to sample identity and treatment when possible. Each experiment will be and include multiple treatment groups. We will test both male and female mice and analyze gender as a variable since SARS-CoV-2 affects men and women differently. Men with COVID-10 die at a greater rate than female patients but it is currently unclear whether disparities in ACE2 expression, innate and acquired immunity or other factors are to blame[81-87]. In contrast, chemosensory deficits appear to be more prevalent in SARS-CoV-2 positive females vs. males[4,88]. Young adult mice (8-10 weeks old) will be used in the proposed studies since determining the role of ACE2 in taste across the lifespan is outside the scope of this 2-year project.

Aim 1. Identify *Ace2* expressing cells in peripheral and central taste pathways as potential targets of SARS-CoV-2.

Rationale In this aim, we will generate *Ace2^{CE}* tamoxifen-inducible CreERT2 knock-in mouse strain by crossing *Ace2^{CE}* founders with wild type C57BL/6J mice. We will then cross *Ace2$^{CE/+}$* mice with conditional tdTomato reporter mice (Ai14 Rosa26-STOP-tdTomato, JAX #007908) to generate double heterozygous *Ace2$^{CE/+}$*, Rosa26-STOP-tdTomato mice. The expression of tdTomato in the *Ace2*-expressing cells will be activated by i.p. injection of tamoxifen (1-5 consecutive daily dosage of 40 µg/g body weight) into adult *Ace2$^{CE/+}$*, Rosa26-STOP-tdTomato mice. The high tdTomato expression level from Rosa26-CAG promoter allows easy detection of *Ace2*-expressing cells. We will co-immunolabel tdTomato and standard taste cell, neuronal, glial and vascular markers to determine endogenous *Ace2* expression in peripheral and central taste pathways listed with abbreviations in Fig. 5: (1) Anterior, posterior and palatal taste buds; (2) Sensory afferent fibers from the CT (anterior), GL (posterior), GSP (palate) and lingual nerves (lingual epithelium); and (3) taste structures from taste ganglia through GC. We will also map SARS-CoV-2 protease activators which promote viral entry to host cells (Fig. 3). Together, these results will indicate potential mechanisms for SARS-CoV-2 induced taste deficits due to the loss of: (1) type I glial-like taste cells; (2) type II sweet, umami, and bitter-sensing cells; and (3) type III sour and salt-sensing cells which also synapse with afferent nerve fibers[89]. Infection of each taste cell population could affect taste qualities directly or through inflammatory bystander effects on intact taste cells. *Ace2* expression in taste stem and progenitor cells suggests that taste cell replacement may also be dysregulated by viral infection[61,63]. *Together results from this aim provide insight to potential targets of SARS-CoV-2 in murine taste buds and central taste regions.*

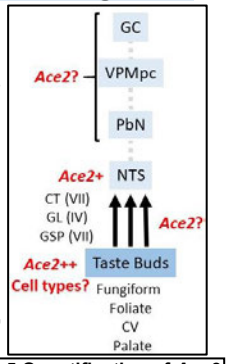

Fig. 5 Quantification of *Ace2* expression in taste structures. *Abbreviations*: circumvallate (**CV**); greater superficial petrosal nerve (**GSP**); glossopharyngeal nerve (**GL**); chorda tympani nerve (**CT**); nucleus of the solitary tract (**NTS**); parabrachial nucleus (**PbN**); medial parvicellular region of the ventral posterior medial thalamus (**VPMpc**); gustatory cortex (**GC**).

Expected outcomes. We hypothesize that: (1) *Ace2* and TMPRSS2 are expressed in type I, II, and III taste cells based on preliminary results (Fig. 2, 3); (2) stem cells in anterior and posterior taste buds express *Ace2* and TMPRSS2[61,63] and (3) taste afferent nerves and their cell bodies are *Ace2* negative based on preliminary transcriptional profiling[63]. ACE2 expression in the NTS is established[90-92] but only preliminary in the PbN to date[93]. To our knowledge endogenous vs. K18-driven[76,94,95] *Ace2* expression has not been determined in the mouse VPMpc or specifically in GC[96-98]. Thus we will confirm Ace2 in the rostral NTS and determine its expression in taste projection areas because their potential for SARS-CoV-2 infection is currently

unresolved[76,86,94,95,99-102]. PbN is not a major taste projection area in humans[103] but its inclusion provides insight to potential RAS regulation of taste and cardiovascular function[90,91,104]. Subsets of endothelial and/or glial cells may express *Ace2*+ though this remains contested and likely varies across brain regions[105,106].

By colabeling with anti-V5 antibody, we can also determine cells that are expressing *Ace2* presently (V5+ and tdTomato) or transiently (V5- and TdTomato+) in taste buds. Furthermore, the highly sensitive cell lineage tracing approach allows the identification of cells with transient and/or weak ACE2 expression, which may not be detected by immunolabeling with ACE2 antibody. These novel ACE2-expressing cells will be the focus of future neurophysiological and behavioral studies using pseudotyped virus.

Immunofluorescence and qPCR. Tamoxifen-induced *Ace2*^{CE}, Rosa26-TdTomato mice and control genotype littermates with and without tamoxifen (n=4/gender/group) will be perfused and taste tissues dissected and cryosectioned. Mouse taste buds will be co-labeled with anti-TdTomato to detect *Ace2*-expressing cells and antibodies to type I marker NTPdase2[107-109], type II marker PLCβ2[110,111], and type III marker carbonic anahydrase IV (CA4)[111-113]. If type II and III taste cells are positive we will co-localize ACE2 with markers for functional subsets (e.g. T1r1, T1r2, T1r3, OTOP-1)[89,114]. We will double label tdTomato with the stem cell markers Lgr6 in anterior taste buds[115], Lgr5 in posterior taste buds[115-117] and the taste progenitor marker K14[117,118]. CT fibers will be identified by P2X3[119-121], cell bodies with Phox2b[122,123], and GL, palatal and lingual nerve fibers with neurofilament[124,125]. TdTomato-expressing *Ace2*+ neurons in the petrosal and trigeminal ganglia and central taste regions will be identified with βIII tubulin[126]. Non-neuronal *Ace2* expression will be co-localized with specific glial[127] and vascular markers[128] since these populations may be targets of SARS-CoV-2[129,130]. Negative control sections will be incubated in normal sera followed by secondary antibodies.

Confocal images will be acquired with a Nikon A1R multiphoton/confocal system and analyzed with NIS Elements Version 4.3 (Nikon) and ImageJ (NIH). Double- and single-positive taste cells will be determined by cell counts or integrated density. Ganglia will be stained as whole mounts and double-positive and single-positive cells counted as described[122]. *Ace2*+ neurons, glia or vessels will be also be quantified in the NTS, PbN, and GC[131-133]. The PIs' department has a new automated analysis system with NeuroInfo software (Microbrightfield) which delineates brain regions on images using the Allen Mouse Brain Atlas[134]. In *Ace2*-TdTomato+ taste structures we will co-localize cell markers and SARS-CoV-2 activators TMPRSS2, furin, NRP-1, and Cathepsin B and Cathepsin L[34,63,65,73,135-137]. We will confirm *Ace*2 and viral entry co-factor gene expression by qPCR in the CV, FG punches, non-taste lingual epithelium and taste neurons in C57BL/6J mice as in Fig. 2-3[138,139]. Single-positive cells, double-positive cells, and mRNA expression will be compared between groups with ANOVAs followed by Bonferroni posttests.

Alternative outcomes and potential pitfalls. Dr. Gan is an expert in CRSPR/Cas9 technology and has extensive experience using Cre-activated reporter gene expression in cell lineage analysis[69,140-143]. We do not expect problems in generating sufficient *Ace2*^{CE} mice. We have validated ACE2 and TMPRSS antibodies in CV and FG taste buds (Fig. 2-3) in case of unforeseen circumstances.

Aim 2a. Determine the contribution of lingual ACE2 to taste function and taste receptor cell dynamics in the healthy and inflamed peripheral taste system. *We will record responses to taste and tactile stimuli from the chorda tympani (CT) nerve and quantify taste receptor cell number and turnover in LE-specific Ace2 knockout mice using a new floxed Ace2 strain. Conditional Ace2 knockout and control mice will also be treated with systemic LPS to test taste function during lingual inflammation.*

Rationale. We will use conditional *Ace2* knockout mice in this aim to probe the function of the SARS-CoV-2 receptor in taste buds. Removing the ACE2 brake on Ang II exacerbates inflammatory responses to LPS and tissue injury in other tissues (Fig. 1). We hypothesize that lingual cytokine and leukocyte responses to LPS will also be elevated in conditional *LE-Ace2* KO mice dampening neural taste responses to multiple stimuli. *The unknown role of ACE2 in taste function is a barrier to understanding taste deficits caused by SARS-CoV-2.*

LPS treatment and neurophysiology. *LE-Ace2 KO* or heterogeneous littermates will receive LPS (5 mg/kg b.w.) or PBS i.p. We will record CT responses to salt (with and without 50 µm amiloride), sweet, bitter, acid, and sour tastants, 4ºC water, and tactile stimulation at 6 and 24 hr after treatment according to standard methods (n=5 male and n=5 female mice/group/timepoint)[138,139,144]. The timing is based on cytokine and neutrophil responses to LPS within hours and changes in taste cell dynamics at 24 hr[54,145-149]. Terminal blood and tissues will be collected following recordings and used to measure circulating cytokine levels with multianalyte ELISAs. Cytokine levels in taste epithelium will also be analyzed by qPCR and local macrophage, T cell and neutrophil responses by immunofluorescence[138,148-151]. We will count proliferating Ki67-positive, apoptotic cleaved caspase-3-positive, and type I, II, and III taste cells in fungiform papillae using confocal

imaging as in Aim 1. The effects of genotype, LPS, gender and time will be identified with mixed-model analyses.

Expected outcomes. Based on widespread ACE2 expression in taste buds (Fig. 2) we expect LPS treatment to suppress responses to multiple taste qualities in *LE-Ace2* KO as taste cell turnover is dysregulated[54,147]. In LPS-treated control littermates, we expect elevated Na^+ responses, inflammatory cytokine levels, and leukocyte responses based on previous results[54,145-149]. We expect *LE-Ace2* KO to exacerbate inflammatory responses to LPS in as in other tissues[31]. A common caveat in the field is that *Ace2* will be deleted in taste cells and lingual keratinocytes because of their shared K14+ lineage[152]. Thus, neural taste changes could be due to taste cell based or indirect mechanisms.

Alternative outcomes and potential pitfalls. The techniques are standard to the field and our laboratories so no difficulties are anticipated. If CT responses to LPS are unaffected by *LE-Ace2* at 6 or 24 hr we will extend survival time with s.c. fluid and record at day 2. However, taste bud levels of the cellular activity marker *c-fos* are suppressed at 6 hr after LPS injection so this is unexpected[147]. ACE2 may play a fundamental role in salt taste in addition to its potential function as a SARS-CoV-2 receptor similar to its dual functions in the lung, kidney, and cardiovascular system[30,31,153,154]. In that case amiloride-sensitive CT responses may be altered in vehicle-treated LE-Ace2 KO.

Aim 2b. *We will record neural responses and measure taste cell dynamics in hAce2-KI which recapitulate endogenous Ace2 expression. Mice will be challenged with LPS and/or a human SARS-CoV-2 spike-Fc fusion protein to determine the impact on taste function.*

Rationale. In this aim we determine the effect of human spike-Fc protein on inflammatory responses, taste bud composition, taste cell dynamics, and neural taste, tactile and temperature responses in mice expressing human *Ace2*. Fc-fusion proteins have improved stability and solubility *in vivo*[155], and this strategy has been successful in demonstrating ACE2-mediated mechanisms of lung injury during SARS-CoV infection[49,51,52]. Results from this aim will contribute to our understanding of how SARS-CoV-2 causes taste deficits and strategies that might be used to treat long-term aguesia.

Methods. CT recordings, LPS injections, analyses of inflammatory responses by ELISA, qPCR, immunofluorescence, taste cell confocal analyses and statistical analyses will be performed as described above. Groups of *hAce2-KI* mice and heterozygous littermates will be injected with LPS or PBS (i.p.). Human spike-Fc or control-Fc protein will be administered i.p. three times at 30 min before, 1 hr after, and 2 hr after LPS injections (n=5 mice/gender)[52]. We will record from the CT and harvest samples at 6 hr after LPS injections since group differences emerged within hours in lung[52]. Antibodies to the receptor binding domain of the human spike protein will be used to measure spike-Fc in Western blots on anterior lingual epithelium and lung lysates and in cryosections from anterior tongue co-labelled with type I, II, and III taste cell markers.

Expected outcomes. We predict that spike-Fc injection will exacerbate inflammatory responses to LPS, reduce taste progenitor proliferation, increase taste cell death, alter the proportion of type I, II, and III taste cells, and decrease CT responses to multiple taste qualities more dramatically than LPS alone.

Unexpected outcomes and potential pitfalls. If taste buds and CT responses are unaffected by LPS plus spike-Fc protein we will extend the survival period to 2 days post-LPS. LPS rapidly affects taste buds[54,145-149], however, and taste deficits appear to be early symptoms of SARS-CoV-2 infection[156]. We could also deliver spike-Fc intranasally and/or i.v., though our goal is to test reactivity to the viral protein rather than immunize mice[157] and human spike-Fc protein was administered systemically in lung models[50,52]. If taste function is unaffected by treatment we would also test commercially-available h*Ace2*-KI mice (JAX #035000).

Timeline

We are breeding mice and will have established breeding colonies by the start of year 1. We will begin Aim 1 and neurophysiological studies for Aims 2a and 2b immediately. We expect to complete Aim 1 by the end of year 1 and CT recordings by the end of year 1.5. Immunological and immunofluorescent assays for Aims 2a and 2b will be ongoing as tissue is added. This allows time to complete all experiments before the end of year 2 even if additional groups surviving 2 days are added.

4. Future Studies

We will test whether taste behavior is altered in *LE-Ace2 KO* and *hAce2-KI* mice receiving LPS and spike-Fc protein with collaborator Dr. David Pittman[138]. A priority is to test whether exogenous ACE2 or the Ang II receptor antagonist, Losartan, ameliorates inflammation, structural changes in taste buds, and neural and behavioral taste deficits. We will also determine whether pseudotyped human SARS-CoV-2 virus, which can be used in BSL2 facilities[63], recapitulates mechanisms underlying taste loss in *hAce2-KI* mice.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

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

VERTEBRATE ANIMALS

1. Description of proposed use of animals.

   Mice. We propose to use n=192 8-10 week old male and female mice of the following strains: (1) *Ace2-P2A-V5-CreERT2* knock-in mice *(Ace2^{CE});* (2) *Ace2* conditional knockout mice (*Ace2^{loxP}*); (3) humanized Ace2 knock-in mice (hAce2-KI); (4) K14-Cre [#018964; B6N.Cg-Tg(Krt14-cre)1Amc/J; and (5) wild-type C57BL/6J mice (#000664). Novel *Ace2* strains will be born and housed at the Medical College of Georgia at Augusta University and the remaining strains will be purchased from the Jackson Laboratory.

   The following animal procedures will be performed by trained personnel:

   a. *Blood collection and i.p. injections*. We will perform terminal bleeds for multianylate cytokine ELISAs. 100 µl LPS or vehicle will be injected i.p. Some mice will also receive i.p. injections of human spike protein.

   b. *Non-survival recording from the chorda tympani nerve*. Mice will be deeply anesthetized with ketamine/xylazine, with supplemental injections given as necessary. Mice are tracheotomized, and the CT approached by a lateral dissection of the head. Sensory responses are recorded as taste stimuli are flowed over the tongue with a syringe. Body temperature is maintained with a water-circulating heating pad for the 1-2 hour recording. At the end of the recording, mice will be euthanized with isofluorane overdose followed by thoracotomy without awakening and tissues harvested for further analyses.

2. Justification. Mice will be used for all aims because of the availability of transgenic strains generated by the Gan Laboratory to determine the expression and functional role of ACE2 in the taste system. The murine immune system is also well-studied and a wide variety of mouse reagents are available. The number of mice is minimized by using them for multiple experimental outcomes where possible. In all aims, mice used for neurophysiological recordings are euthanized then tissues harvested for immunofluorescence and qPCR. We will also work with Laboratory Animal Services to share tissues and animals with other PIs on campus when possible. Animal studies are needed because we are investigating complex processes in the taste-immune axis which cannot be adequately modeled *in vitro* at this time.

3. Procedures to limit discomfort, distress, pain, and injury. Surgeries to dissect and record from the chorda tympani nerve will be performed under deep anesthesia to prevent pain and distress. Mice are euthanized following recordings before awakening. Dr. McCluskey has >20 years of experience recording sensory responses in rats and mice. All laboratory personnel are trained to recognize pain and distress in mice.

4. Euthanasia. Euthanasia will be accomplished by methods consistent with the AVMA Guidelines for the Euthanasia of Animals.

MULTIPLE PI LEADERSHIP PLAN

Rationale for multiple PI approach. This application has two PIs: Dr. Lynnette McCluskey and Dr. Lin Gan, who are both faculty in the Department of Neuroscience & Regenerative Medicine at the Medical College of Georgia at Augusta University. Dr. McCluskey's group has studied neural-immune interactions in the taste system for over 20 years. She has expertise in peripheral taste regulation by the immune system using molecular, cellular, immunological and neurophysiological methods. Dr. Gan's lab studies sensory cell loss, neuronal differentiation, circuitry formation and neurodevelopment. He has extensive experience in mouse genetics and is the Director of the departmental Transgenic and Genome Editing Core Facility. The PIs have adjacent offices, laboratories on the same floor, and developed this R21 proposal after discussing newly generated ACE2 mouse models, sensory deficits, and the urgent need to determine mechanisms underlying SARS-CoV-2-induced taste loss.

Governance, organizational structure, and responsibilities. Dr. McCluskey will oversee the administration of the grant and serve as the contact PI. She will manage her personnel and collaborators and oversee compliance (i.e. mouse IACUC and biosafety protocols) to complete studies in Aims 1 and 2. Dr. Gan will provide the *Ace2* mouse models and perform genotyping and histology.

Plans for communication. The proximity of our adjacent offices and laboratories on the same floor ensures that communication will be frequent. Joint laboratory meetings with all laboratory personnel and Dr. Linder will be held monthly. Consultants will be briefed on results and assistance requested as needed. Data files will be shared, updated, and continually available to both PIs via Box software.

Authorship and resolution of conflict. We do not anticipate any disagreement on scientific direction or authorship based on our collegial relationship since Dr. Gan's arrival at the Medical College of Georgia. In the unlikely event that there is a significant disagreement PIs will submit to independent arbitration by a neutral party such as an AChemS officer.

Authorship will be determined by the Society for Neuroscience (SFN) guidelines for authorship. We expect that Dr. McCluskey will be senior author on publications since the project is focused on the taste system which is her area of expertise, though in some cases senior authorship will be shared. The first author will be determined by the significance of contribution and may be a PI or member of either lab. All undergraduate students, graduate students and lab personnel at Augusta University or Stetson University making significant contributions to the research as defined by SFN will share co-authorship.

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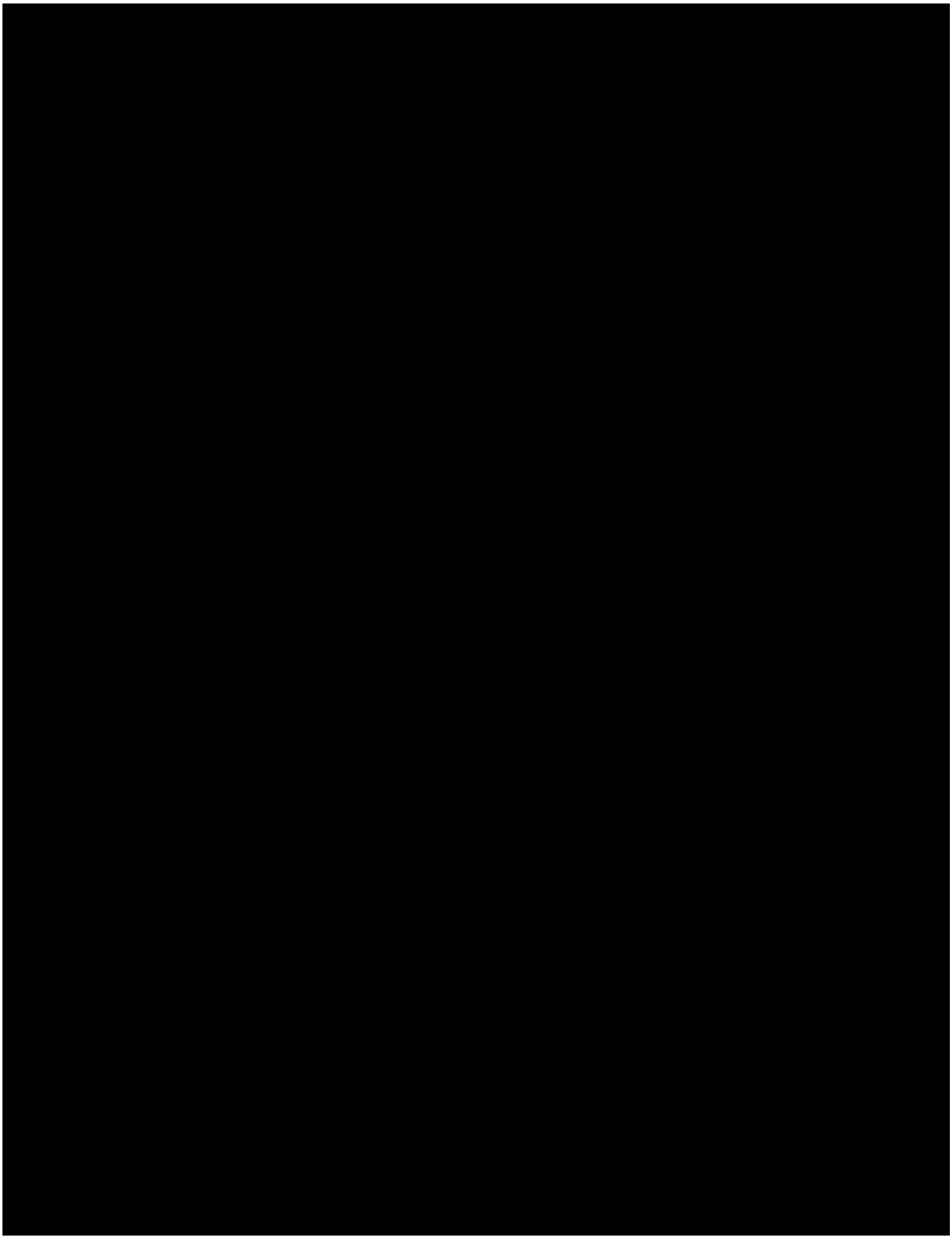

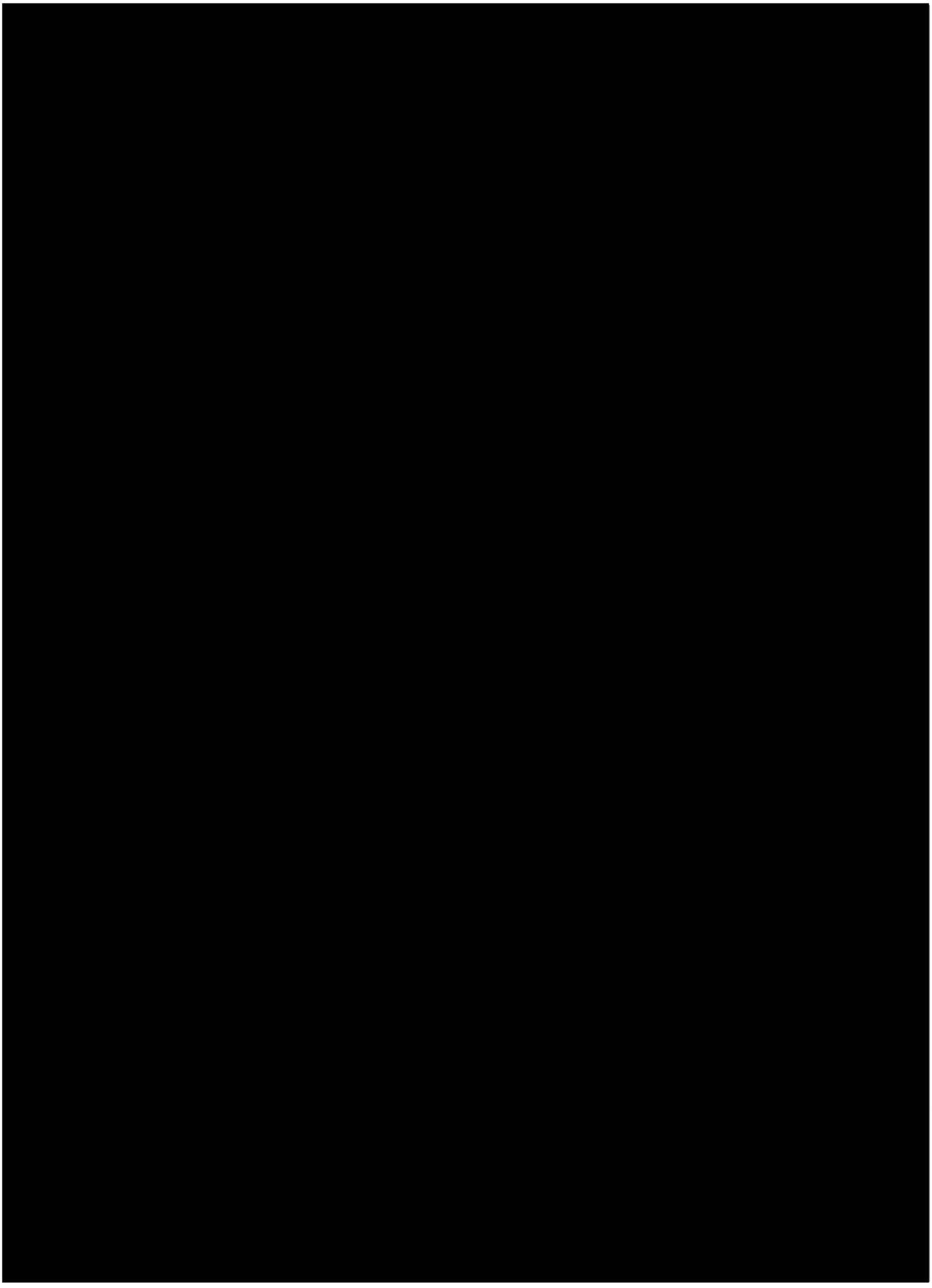

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RESOURCE SHARING PLAN

We will adhere to the NIH Grants Policy on Availability of Research Results: Publications, Intellectual Property Rights, Sharing Biomedical Research Resources and Sharing Model Organisms for Biomedical Research.

We will make available any protocols, reagents or requested designs. Specifically, sharing of research materials generated in this project will be made under terms no more restrictive than in the Simple Letter Agreement for the Transfer of Materials or the Uniform Biological Materials Transfer Agreement (UBMTA), and made without reach-through requirements. We will make it available to the research community any reagent or resource that is presented in a publication that is published or accepted for publication from our laboratories. We agree to release and share data/results/findings in a timely manner, with the date of release to be no later than acceptance for publication of our main findings from our final data set. All raw and processed data contributing to publications arising from this work will be packaged into well-documented datasets and distributed through public repositories.

More specifically:

A. Molecular tools
   Plasmid constructs generated by this project will be distributed freely or deposited into Addgene to make them available to broader research community, either before or immediately after publication.

B. Model organisms:
   All new mouse lines generated in this study will be either deposited to The Jackson Laboratory or shared with individual investigators upon request after publication.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

1. Antibodies. Each antibody will be purchased from reliable sources, and will have been cited in multiple publications and/or high-quality publications by colleagues. Antibodies used as taste cell and leukocyte markers have been used extensively in multiple publications from our laboratory and/or many others. Immunologist colleagues at the Medical College of Georgia are readily available for consultation and positive control samples as needed. Careful records of lot numbers and assay outcomes are kept in the laboratory. Our typical immunohistochemical controls include: omission of primary antibody, incubation in matched concentrations of preimmune sera and pre-adsorption with excess antigenic peptide and for newer antibodies Western blotting to test specificity (Shi et al., 2012).

2. Chemicals and Biologicals. We will use fresh stocks of E. coli 0111:B4 LPS (5 mg/kg; Sigma) diluted in sterile, endotoxin-free water as in our previous work. Human spike-Fc fusion protein (R&D Systems) has been widely tested since the earlier SARS-CoV pandemic. Mice will be injected i.p. three times with spike-Fc or control-Fc (5.5 nmol/kg) diluted in sterile, endotoxin-free water (Kuba et al., 2005). Spike-Fc binding is validated with His-tagged human ACE2 in functional ELISAs and pull down assays. Elevated levels of spike-Fc will be confirmed in taste tissues and lung by Western blot and immunoassays to co-localize with taste cell markers. Taste solutions are freshly made weekly and stored at 4° C between recordings before warming to room temperature. Chemicals are purchased from Sigma. All other chemicals and biologicals have been verified in our groups' prior research and in publications.

3. ELISAs. We have used multi-analyte and single-analyte ELISAs from Qiagen and R&D Systems for many years with good inter-assay and intra-assay reliability. Blood is collected at the same time of day to control for circadian influences in cytokine assays. All chromogenic assays are performed in duplicate or triplicate. Recombinant mouse cytokines that serve as kit positive controls have shown strong responses, while negligible optical density values are generated by negative wells. We also include sera, plasma, tissue lysates, or conditioned media from positive and negative controls.

4. Primers. We will use primer pairs as published by multiple sources in the field and check specificity with Primer Blast. Primers that span an intron will be chosen when possible to avoid detecting contaminating genomic DNA.

5. Animals. Health reports from Jackson and from the MCG-AU mouse facility are monitored to ensure that mice are free from specified pathogens. Humidity, temperature and light cycles are controlled within the barrier animal facility.