

SUMMARY STATEMENT
(Privileged Communication)

Release Date: 11/22/2021
Revised Date:

PROGRAM CONTACT:
[REDACTED]

Application Number: 1 R21 DC019832-01A1
Formerly: 1R21DC019832-01

Principal Investigators (Listed Alphabetically):

GAN, LIN
MCCLUSKEY, LYNNETTE MARIE (Contact)

Applicant Organization: [REDACTED]

Review Group: NIC
Neuroscience of Interoception and Chemosensation Study Section

Meeting Date: 10/28/2021
Council: JAN 2022
Requested Start: 04/01/2022

RFA/PA: PA20-195
PCC: CS04

Project Title: Ace2 in the healthy and inflamed taste system

SRG Action: Impact Score:17 Percentile:3 +
Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm
Human Subjects: 10-No human subjects involved
Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

Project Year	Direct Costs Requested	Estimated Total Cost
1	[REDACTED]	[REDACTED]
2	[REDACTED]	[REDACTED]
TOTAL	[REDACTED]	[REDACTED]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

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RESUME AND SUMMARY OF DISCUSSION: The proposed exploratory research project is designed to test the hypothesis that taste buds are SARS-CoV-2 targets, that 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. The reviewers agreed that insight into mechanisms responsible for taste dysfunction due to SARS-CoV-2 are likely. All of the needed expertise and infrastructure are in place and additional collaborators have been recruited to the project to help advise portions of the study that require analysis of neuroanatomy of taste-related areas of the PNS and CNS. The questions are timely and important, novel transgenic mice will be utilized and the response to the prior review was thorough, including an additional transgenic mouse line. Rigor of the prior research was well-addressed, including discussion of gaps in the current literature, the experiments are well-designed and controlled, and both male and female mice will be studied. All in all, the possibility of attaining a better mechanistic understanding of how SARS-CoV-2 interacts with the taste system is strong, which is highly significant.

DESCRIPTION (provided by applicant): 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.

PUBLIC HEALTH RELEVANCE: 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.

CRITIQUE 1

Significance: 1

Investigator(s): 1

Innovation: 1

Approach: 2

Environment: 1

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Overall Impact: This is a revision of an R21 multi-PI proposal from Lynette McCluskey, an investigator with established expertise in taste-immune interactions. She has established a collaboration with Dr. Lin Gan, a developmental biologist with expertise in manipulating the mouse genome to develop three novel models appropriate to address critical questions about interactions of the SARS-CoV-2 virus with the taste system. The experiments will (1) probe expression of ACE2 and TMPRSS2 throughout the gustatory neuraxis, (2) explore the function of endogenous murine ACE2 in peripheral taste responsiveness and (3) Use a humanized ACE2 knock-in model to assess if the SARS-CoV-2 spike protein interferes with normal taste function. The questions are timely and important. The mouse lines avoid problems presented by currently available models and have been revised in line with the previous reviews. Two of the three have already been developed. The team and environment are excellent, and the revision brings in extra consultants for analyzing the CNS data, as suggested by previous reviewers. Additional preliminary data partially allays previous concerns regarding the specificity of immunostaining. Enthusiasm is high.

Rigor of the Prior Research: The literature indicates considerable uncertainty with regard to the prevalence of ACE2 and TMPRSS2 expression in taste buds; these studies are cited by the PI and represent a gap to be filled by the proposed research. The PI cites a study by Doyle et al., as evidence for ACE2 expression in human taste buds. That study is not very convincing.

Level of Experimental Rigor in the proposed research project: Each experiment uses appropriate controls, e.g., including blinded analyses of anatomical data. The previous reviews questioned the specificity of the immunohistochemistry in the preliminary data and noted that there were no negative controls omitting the primary antibody. These images have been added and show a lack of staining. Nevertheless, the high level of staining and its apparent ubiquitous distribution in the bud remains surprising. However, the proposed studies should resolve remaining questions, at least for ACE2 staining.

Sex as a Biological Variable: Both male and female mice will be used in all studies and the possibility of differences based upon clinical observations is discussed.

1. Significance:

Strengths

- Covid-19 has been associated with chemosensory deficits. Olfactory deficits are the best documented but taste deficits are also possible, perhaps likely. These experiments will perform critical experiments for establishing a cellular basis for these deficits.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Dr. Lynette McCluskey is a productive, established investigator. She is one of a small cohort of scientists who have focused on taste-immune interactions.
- Dr. Lin Gan is a developmental biologist who has made several significant discoveries in the molecular basis of retinal and inner ear development. He has long-standing expertise in manipulating the mouse genome and is director of the Transgenic and Genome Editing Core at Augusta University, Medical College of Georgia.

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- The other members of the team are likewise excellent, provided needed expertise in all aspects of the project: CT nerve recording (G. Dong), confocal imaging/immunoassays (S. Kogan), and statistical support (D. Linder).
- In response to previous concerns that the team did not have expertise in CNS anatomy, additional members have been added to the team: Two individuals with established expertise in neuroanatomy, Camille King & Michael King will consult on the gustatory CNS mapping of ACE and Xin-Lun Yu will provide complementary expertise for central regions outside the gustatory system.

Weaknesses

- None

3. Innovation:

Strengths

- Novel mouse models relevant to Covid-19.

Weaknesses

- None

4. Approach:

Strengths

- The proposed mouse models study the expression and the effects of deletion and humanization of the ACE2 receptor under control of the endogenous promoter.
- Two of the three mouse models have been developed and validated with PCR.
- Elucidating the expression of ACE2 throughout the gustatory neuraxis using a conditional tdTomato reporter under the control of the endogenous promoter will greatly clarify the location of this receptor. These experiments have potential to extend beyond the gustatory system.
- Recording experiments in the mouse that express the humanized ACE2 receptor to probe the effects of the Spike protein should be particularly revealing.
- Sex as a biological variable is well-considered as are standard considerations for rigor.

Weaknesses

- Minor: Although the addition of negative control data for immunostaining is an improvement, the strong and ubiquitous staining for both the ACE2 and TMPRSS antibodies still raises some doubts. It is appreciated that the reporter mouse for ACE2 will help to resolve this question. However, the location of TMPRSS may still be in question and additional controls to check the antibody specificity would be appropriate. Overall, more skepticism about these data would have been reassuring.

5. Environment:

Strengths

- Excellent facilities and a supportive environment.

Weaknesses

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- None

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all criteria addressed

- All the criteria are carefully & comprehensively addressed.

Biohazards:

Not Applicable (No Biohazards)

Resubmission:

- Addressed above.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2

Significance: 1

Investigator(s): 1

Innovation: 2

Approach: 2

Environment: 1

Overall Impact: This revised R21 by PIs McCluskey and Gan proposes to study the role of ACE2 in the peripheral taste system and whether ACE2 when bound to SARS2-spike protein has a detrimental effect on TRCs and taste function. This is a very timely and important study given the world-wide impact of COVID and potential lingering taste loss after acute disease recovery. This proposal uses newly engineered mouse knock-in strains to label Ace2 expressing cells and also to introduce humanized Ace2 into the endogenous mouse locus to better understand the effects of SARS2 spike protein on the taste system. A floxed conditional knock-out strain will also be used to study the contribution of Ace2 in normal and inflamed taste tissues. The investigators have all of the expertise required to perform these studies, and these new strains, once characterized fully could be of great

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utility to the field (although there is already a humanized KI strain available). PIs have been very responsive to prior reviewer comments, changing the strategy to use a V5 epitope tag rather than a GFP for co-labeling (a nice improvement). The PIs have also recruited other collaborators to assist in the anatomical mapping of ACE2 expression throughout from the periphery to the CNS.

Rigor of the Prior Research: Early studies of ACE2 expression within taste cells was not very vigorous, but now this is well established in both human and mouse taste tissues.

Level of Experimental Rigor in the proposed research project: High

Sex as a Biological Variable: Both sexes included, and sex will be used as a biological variable since COVID affects males more than females. ACE2 is located on the X chromosome – but this was not mentioned in the research strategy, which may play a role in sex differences in the engineered mice.

1. Significance:

Strengths

- The widespread and long-term effects of COVID is a significant problem facing the world today. Understanding how SARS-coV2 infection affects the taste system may reveal ways to combat taste loss in patients.
- Understanding the normal role of ACE2 in the taste system is an important goal itself.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- PI McCluskey is perfectly suited to lead these investigations having a distinguished record of studying the interactions of inflammation and immunology in the taste system.
- PI Gan is the director of the transgenic core at Augusta and has all of the expertise necessary to engineer the transgenic mouse lines for these experiments.
- Collaborators have been recruited to the project to help advise portions of the study that require analysis of neuroanatomy of taste-related areas of the PNS and CNS.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The transgenic mouse lines developed here are elegantly designed and would be very useful for others in the field.

Weaknesses

- One of the lines – the humanized knockin already has been developed and is available at JAX. Is this a duplication of effort, or is there a rationale for developing this line to overcome some weakness of the currently available strain?

4. Approach:

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Strengths

- Each aim has a straightforward approach and a well-justified rationale.

Weaknesses

- All of the aims depend on the success of the transgenic lines. Given the skill and expertise of the PIs, I am confident that these lines will indeed be successfully generated and will allow the experiments to be completed as planned.

5. Environment:**Strengths**

- Excellent environment to successfully complete this work.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all criteria addressed

- Acceptable

Biohazards:

Not Applicable (No Biohazards)

Resubmission:

- PIs have been very responsive to previous critiques.

Resource Sharing Plans:

Acceptable

- Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

MCCLUSKEY, L

Significance: 2
Investigator(s): 2
Innovation: 3
Approach: 3
Environment: 2

Overall Impact: This is an exciting resubmission from a highly productive (even in pandemic times) researcher from Augusta University, who proposes to study the role of ACE2 receptor in taste responsiveness. This effort has both basic and translational arms, in that ACE2-R is involved in COVID-19 infection, and the investigator will examine how the presence of the spike protein impacts peripheral taste function. Thus, this research holds the promise of both aiding in our understanding of peripheral taste function and potentially providing the beginnings of an explanation of COVID-related perturbations of that function. Furthermore, the investigator has been satisfyingly responsive to the prior critiques, making major changes and bolstering questionable aspects of the proposal. An eminently worthy proposal.

Rigor of the Prior Research: Extremely high

Level of Experimental Rigor in the proposed research project: Great, and even better in the resubmission.

Sex as a Biological Variable: handled.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMALS: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R21 DC019832-01A1; PI Name: MCCLUSKEY, LYNNETTE Marie

+ Derived from the range of percentile values calculated for the study section that reviewed this application.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-18-197 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Neuroscience of Interoception and Chemosensation Study Section
Integrative, Functional and Cognitive Neuroscience Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW

NIC

10/28/2021 - 10/29/2021

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html>, NOT-OD-15-106 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html>, and NOT-OD-18-115 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-115.html>, including removal of the application from immediate review.

CHAIRPERSON(S)

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