Effects of Nerve Transection on Taste Nerve Function

Hypotheses:

H_1: Chorda tympani nerve response to sodium chloride will be weaker in animals from the surgical group than those in the control group following lingual nerve transection.

H_2: No difference in chorda tympani response to sucrose, citric acid, or quinine will be observed between the surgical and control groups following lingual nerve transection.

H_3: The amiloride sensitive response to sodium chloride will be weaker in animals from the surgical group than those in the control group following lingual nerve transection.

Project Description:

The peripheral nervous system includes nerves that are responsible for transmitting information from different senses (e.g., auditory, visual, taste, etc.) to the brain. In much of the mammalian system, different peripheral nerves are specific to different peripheral organs. For example, visual information is detected by the eye and processed by the optic nerve while information about sound is detected by the ear and then processed by the auditory nerve. However, the tongue houses two distinct nerves responsible for the transmission of two distinct sensory modalities: taste (gustatory) and touch/temperature (somatosensory). This presents an interesting model to assess whether individual peripheral nerve function depends on the proximity, activity, and status of other nerves.

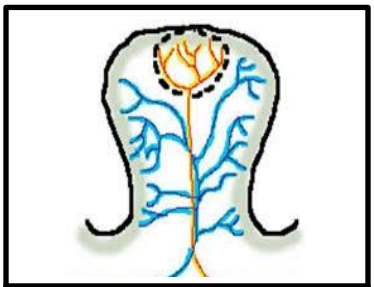

Figure 1. Chorda tympani innervation (orange) and lingual nerve innervation (blue) within the taste bud and papilla respectively (Omelian et al., 2016).

The chorda tympani nerve (CT) projects to the taste buds of the tongue which detect taste information (sweet, salty, bitter, sour, and umami compounds) that the CT sends to the brain (Figure 1; Miller et al., 1978). This common reaction is vital to determining what substances are safe for consumption (Rozin, 1976). In addition to taste, somatosensory information is also relayed by peripheral nerve function. Larger structures known as papillae encapsulate the taste buds and are responsible for detecting somatosensory stimuli. Papillae cover the surface of the tongue and are directly innervated by the lingual nerve (LN; Figure 1), which is responsible for sending somatosensory information to the brain (Farbman & Mbiene, 1991).

Both the CT and the LN are necessary features of the gustatory and somatosensory systems and have been found to depend on one another in certain contexts for the maintenance of their associated tissue. The consequences of cutting (transecting) these nerves have increased understanding of this relationship (Sollars & Hill, 1998; Sollars & Bernstein, 2000; Sollars et al., 2002; Hendricks et al., 2002; Sollars, 2005; Guagliardo & Hill, 2007; Martin & Sollars, 2015; Omelian et al., 2016). Rats that undergo either CT transection (CTX) or LN transection (LX) experience a decrease in taste bud volume as well as a deleterious change to the structure of their papillae (Sollars & Bernstein, 2002; Sollars, 2005; Omelian et al., 2016). When CTX is performed earlier in development, the observed effects are more severe and persist throughout 50 days post-surgery (Sollars, 2005). In contrast, the effects of developmental LX are transient, with a comparatively minor reduction in taste bud volume being the only long-term effect (Omelian et al., 2016). In short, damaging one nerve causes deterioration of the tissue associated with the other nerve.

This codependent relationship has been further illuminated by the assessment of electrical signals sent by these nerves in response to taste solutions. The CT of rats is particularly responsive to sodium chloride (Frank et al., 1983). After CTX in young, developing rats, overall response to sodium chloride in other gustatory nerves decreases when they reach adulthood. No such change in the responses to other taste solutions such as sucrose, citric acid, and quinine has been indicated after CTX (Martin & Sollars, 2015). Some chemical compounds block responses to specific kinds of gustatory stimuli. The drug

amiloride for example stops the CT from transmitting sodium chloride signals (DeSimone & Ferrell, 1985). However, this blockage is not entirely comprehensive, as there are two pathways within the CT capable of transmitting sodium chloride (Halpern, 1998). One is not affected by amiloride (amiloride-insensitive) which accounts for the small but present signal following application of the drug (Lewandowski et al., 2016). The amiloride-sensitive response is greatly affected by the drug and is even weaker after adjacent nerve transection compared with control groups (Martin & Sollars, 2015). By focusing on the effects of the sodium chloride/amiloride interaction, the target pathway within the CT can be identified and greater specificity is achieved.

Given that the LN and CT are close in proximity and that LX decreases the volume of CT-innervated taste buds, LX is likely to change CT activity. A relationship between the electrical activity of the LN and CT has already been indicated (Wang et al., 1995), but how the activity of the CT nerve depends on the functional status of the LN is yet undetermined. To address this, the LN will be transected and the CT response to various taste solutions (including sodium chloride in the presence of amiloride) will be observed.

This research is highly relevant to minimizing major gaps in knowledge about sensory system interactions. There are also implications for improving recovery after LN damage sustained during dental procedures. This outcome is not uncommon and occurs following extraction of the mandibular third molar, a frequently performed surgical procedure (Tojyo, et al., 2019).

Methodology:

Experimental Design

The current study will observe CT signals after both LX and control surgeries in female Sprague-Dawley rats (n = 5/condition, 10 total). Control surgeries will follow the same procedures as the LX surgery to factor out treatment-related variation but will only access and observe the nerve, rather than transect it. All rats will undergo either LX or control surgery at 10 days of age. This period of early development is characterized by a more profound rate and severity of taste bud volume change after LX. The CT signals of all groups will be measured 50 days following surgery, corresponding to the previously established timeline of taste bud and papillae degeneration (Omelian et al., 2016).

Techniques

Various taste solutions (sodium chloride, sucrose, citric acid, and quinine) will be applied to the tongue of each rat in order to stimulate the CT. To determine how effective LX is at changing CT responses, each solution will be applied at various concentrations (0.001 M - 1.0 M). Responses to the stimuli will then be measured and recorded. In line with prior electrophysiological studies, a standard taste solution (0.5 M ammonium chloride) will be applied before and after each series of concentrations. The responses to ammonium chloride serve as a set activity profile to compare with other responses and will ensure the viability of CT responsivity (Sollars & Hill, 1998). To measure these signals, an electrode will be placed on the CT. Distilled water will be applied to the tongue to rinse out taste solutions in between recordings. After responses to the previously mentioned taste solutions are recorded, amiloride will be placed on the surface of the tongue (Martin & Sollars, 2015). After the blocking of amiloride-sensitive pathways is ensured, a mixed solution of amiloride and sodium chloride will be applied to the tongue at the same concentration levels as those used before amiloride application. The CT response will then be recorded. An average of the responses to each solution relative to the responses to the standard solution (ammonium chloride) will be calculated and compared within groups. Repeated measures ANOVA will be conducted to compare the average response to different taste solutions between surgical conditions. Any statistically reliable findings will be assessed using post-hoc analysis with an independent t-test.

Final Product of Funding:

This project will provide material for a presentation at the UNO RCAF and my master's thesis. I anticipate a peer-reviewed publication will result. The research will provide excellent exposure to the techniques of electrophysiology and supplement data that will function as the foundation of my future research.

Potential Pitfalls:

It may be the case that taste response testing at 40 days after LX may not be a long enough post-surgical time to detect significant changes to CT activity. Previous research detected taste response changes in nearby gustatory nerves after a longer period of time post-CTX (Martin & Sollars, 2015). However, in my proposed study, the CT and LN are in much closer proximity to one another than the associated nerves in the Martin & Sollars study. Additionally, our lab has noted anatomical changes to taste buds and papillae at 40 days post-LX (Omelian, et al., 2016) suggesting the 40-day-post time frame of neural recording in this proposal is appropriate. However, to prioritize caution and efficiency, pilot animals (n = 2) will be used to ensure change in activity can be measured at the time points of interest. If no change is detected, the study will be altered and conducted to accommodate for various testing times. The pilot data will serve the purpose of identifying the time frame in which LX impacts CT activity and can be useful (in conjunction with other studies) at generating future research.

Project Timeline:

Date	**Objective**
February - April 2022	Order supplies/practice electrophysiological recordings; make solutions
May 2022	Rat breeding
June 2022	Primary LX and control surgeries
July - September 2022	Post-surgery and control electrophysiological measures (50 days post-surgery)
October - November 2022	Complete analysis and statistics of recordings
December 2023 - February 2023	Finalize results
March 2023	Present results at RCAF

A) Student/Faculty Mentor Roles

I, Nicholas Weber, will be responsible for electrophysiological recordings and data analysis. Dr. Sollars will meet with me regularly to consult on the project, help troubleshoot if problems arise, and perform the LX surgeries. I will also present ongoing results to the laboratory group to get feedback and discuss progress.

Budget Justification

In addition to the materials used for my project, I am requesting a stipend of $3,395.00. The proposed study will take an estimated 400 total hours and the funds will serve to provide me with living expenses during that time. Dr. Sollars and the Psychology Department will provide all other supplies and excess costs. I, Nicholas Weber, am currently a UNO teaching assistant for the Fall 2021 and Spring 2022 semesters.

Item	**Cost**
Solution Chemicals (Citric Acid, Ammonium Chloride; VWR)	$280
Anesthetic (Ketamine, Xylazine, Methohexital Sodium; Covetrus, Inc.)	$725
Animals (6 Male and 8 Female Sprague Dawley Rats; Charles River Labs)	$600
Total Requested for Supplies	**$1605.00**
Requested Stipend	**$3395.00**
Total Requested	**$5000**

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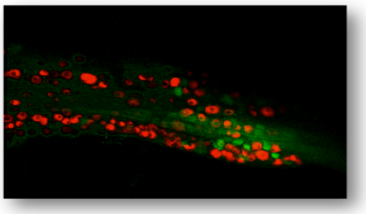

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January 24, 2022

Dear GRACA Selection Committee:

I am providing my strongest recommendation and support for Nick Weber's GRACA application. Nick's proposed experiment will lay the groundwork for furthering his graduate studies by accomplishing a primary study within his Master's Thesis. With the information derived from his experiments in this GRACA proposal, he will have data to present at the Research and Creative Activity Fair, national conferences in neuroscience, and have a key dataset leading to a peer-reviewed publication. The foundation of the work is a component of my laboratory, but the work will be Nick's and separate and unique from other ongoing studies in the lab. Nick will be first author on any conference proceedings or papers that result from these experiments.

Nick's proposed experiments are well designed and can be accomplished within the proposed time frame. The data he obtains will be novel to the field of sensory system neuroscience and of great interest to the scientific community because it addresses not only sensory function but is also relevant for scientists who study developmental neuroscience, neural injury effects, and regeneration. It has an interesting angle that will be especially interesting to the dental community, since it deals with nerves that are often negatively affected by dental surgeries.

The budget is aligned well with the requirements of his GRACA experiments. We have many of the supplies beyond those in the budget and have all of the equipment that is needed to accomplish the work. My lab will be able to provide any items necessary that are not in the proposed budget. As Nick's graduate advisor, I will meet with him on a regular, weekly or biweekly basis to discuss the progress of the experiments, help with data analysis, and provide any support that may be needed to accomplish the project. In addition, my lab has weekly lab meetings where Nick will report on study progress to other graduate and undergraduate students in my laboratory.

I am well-versed in the techniques Nick will be using to record neural activity, so I will be available to train and assist where needed. My role in the experimental process will be to do the initial live animal neural transection surgeries (LX), as these procedures take months to learn and I have years of experience in doing the surgeries. Nick will learn the electrophysiology surgery and independently acquire the neural recordings. Nick has a solid research background for conducting the proposed studies, though because of the pandemic, our ability to conduct live animal research has not been fully active until recently. He is anxious to be able to build his data set for his Master's thesis with the studies in his GRACA proposal.

I am enthused for Nick to start work on this project. It will result in novel information and garner widespread interest in the scientific community. These studies will provide Nick with a strong foundation to develop further studies for his next steps as a PhD student.

Sincerely,

Suzanne Sollars, PhD
Director, Program in Neuroscience
Professor, Psychology and Neuroscience