Procedural Synopsis for the Assessment of the Neurobiological Parosmia Mechanism

Josiah B. King ’23 and Michael A. Berberoglu

Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943

Abstract

The long-term effects of Covid-19 are still heavily understudied. Covid often leaves a long-lasting effect on the olfactory epithelium, inducing a woeful symptom known as parosmia. Parosmia is essentially a distortion of smell and taste. Previously pleasant tastes and smells become unrecognizably foul to victims of parosmia. Among the long term Covid symptoms, parosmia is the most common. Covid induced parosmia is most prevalent in young adults. Women also are more likely to experience covid induced parosmia than men. Even though there is a surplus of parosmia victims, research on the covid induced symptom is extremely underperformed. This is undoubtable partially due to the high degree of difficulty involved in research that involves olfactory and neurological systems. My goal is to make this process easier and encourage more meaningful research on parosmia.

This research serves as a preface for parosmia experimentation and other procedures dealing with olfactory epithelia. Current research concludes that the parosmia mechanism involves neuroinflammation and the distortion of gray matter (Distadio et al. Parker et al. Lafreniere.) This research essentially starts to help the scientific community understand this neuroinflammation and gain new useful understandings of the understudied mechanisms. If achieved, this type of understanding would allow the scientific community to use the zebrafish’s olfactory system as a controllable system. This controllable system could be used as an experimental model for learning more about parosmia and other types of olfactory related mechanisms in humans. The methods used involve staining, immunohistology and polymerase chain reaction. The methods described here are simply peripheral experiments intended to provide general yet essential information. This includes quantifying and locating important cells and genes and also comparing results to human models. Ultimately, this research will aid in the creation of an easily accessible in-vivo parosmia model due to the fact zebrafish are rather inexpensive testing subjects and they express a higher rate of regeneration than most other similar organisms. This means that experiments can be completed and observed at a faster rate.

Ways to Analyze Zebrafish Olfactory Tissues

Immunology

This involves the use of an antibody that is created specifically for the desired ligand or receptor. The antibody then can only bind to the desired protein. The antibodies used also include a fluorescent factor in attempt to make quantifying the desired ligand or receptor easier.

Cell staining

Cell staining procedures are fairly straight forward. Different cells can be stained differently due to unique attributes such as a thick cell wall.

Polymerase chain reactions

PCR or Polymerase chain reactions is essentially the process of exponentially replicating strands of DNA in order to locate DNA mRNA or RNA

Aspects of Olfactory Tiisue to be Analyzed

<u>*ACE2*</u>

ACE2 helps regulate blood flow in the renin angiotensin system along with blood pressure and heart function. It also is Covid-19’s primary binding site. A Covid infect has been reported to cause neuroinflammation. This neuroinflammation is the common factor in this line of research because not only does ACE2 play a large role in regulating inflammation in the body but parosmia is presumably induced by neuroinflammation. ACE2 is also relatively abundant on neural tissues.

<u>*dACE*</u>

dACE is a gene that is activated through INF signaling and the JAK/STAT pathway. It controls the amount of ACE2 upregulation depending on the tissue and how it is activated (Jankowski et al.).

Both ACE2 and dACE2 are to be quantified in our zebrafish model using PCR.

<u>*Cysteinyl Leukotriene Signaling*</u>

Similar to the renin angiotensin system, the cysteinyl leukotriene system helps regulate bronchoconstriction, vascular permeability, eosinophil recruitment, and chronic inflammation. It is a G-protein mediated system. Research suggests that cysteinyl leukotriene signaling also plays a role in the regeneration and the remodeling of airways. Leukotriene C4 (LTC4) is a CysLT1 ligand that enhances CysLT1 without causing an inflammatory response when injected into the ventricle of the zebrafish brain without lesion. Neuronal tissues often lack enzymes to produce LTC4. Cells such as these relies on surrounding Neutrophils to catalyze the reaction. Therefore, within our zebrafish model it is important to quantify and locate the ligand LTC4 and neutrophils with the use of florescent antibodies.

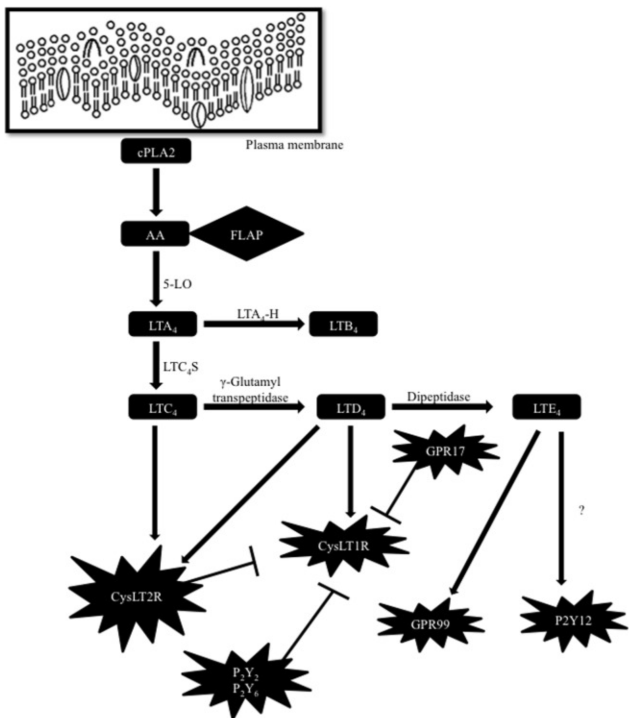

Figure 1: A visual diagram of cysteinyl leukotriene signaling (Thompson-Souza GA)

Wrapped and Unwrapped Olfactory Receptor Neurons

The olfactory receptor neurons are often wrapped by olfactory sustentacular cells. The wrapping is hypothesized to have a myelin-like effect on the olfactory receptor neurons. This ties in with Covid induce parosmia because it has been reported that Covid-19 targets the cells that support the olfactory nerves such as the sustentacular that often wrap the olfactory neurons.

Wrapped and unwrapped can be quantified using Tuj1 immunoreactivity. Correlation of Tuj1 immunoreactivity intensities with wrapping status of ORN dendrites revealed that essentially all enwrapped dendrites have weak Tuj1 immunoreactivity. Strong Tuj1-immunoreactive ORN dendrites are mostly located at the inter-sustentacular cell borderlines, and thus belong to the unwrapped subtype. A small number of the partially wrapped dendrites also exhibit high Tuj1 immunoreactivity (Liang).

Future Work

This research should be a foundation to build on. With an idea of some of the cells within the zebrafish olfactory, the next steps should be to test the function of each individual biomolecule in the zebrafishes' olfactory epithelium. To do this one must knock out or render a cell or system nonfunctional the analyze what behavioral or regenerative properties become impaired.

REFERENCES

A. Di Stadio, L. D'Ascanio, I. La Mantia, M. Ralli, M.J. Brenner. Parosmia after COVID-19: olfactory training, neuroinflammation and distortions of smell. *Eur Rev Med Pharmacol Sci*. 2022 Jan;26(1):1-3. doi: 10.26355/eurrev_202201_27739. PMID: 35049034.

Choi J, Azmat CE. Leukotriene Receptor Antagonists. [Updated 2022 May 30]. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554445/

Huang, Wei-Chang et al. "Treatment of Glucocorticoids Inhibited Early Immune Responses and Impaired Cardiac Repair in Adult Zebrafish." *PloS* one vol. 8,6 e66613. 21 Jun. 2013, doi:10.1371/journal.pone.0066613

Hou, Yujun et al. "NAD+ supplementation reduces neuroinflammation and cell senescence in a transgenic mouse model of Alzheimer's disease via cGAS-STING." *Proceedings of the National Academy of Sciences of the United States of America* vol. 118,37 (2021): e2011226118. doi:10.1073/pnas.2011226118

Jankowski, Jakub et al. "Interferon-regulated genetic programs and JAK/STAT pathway activate the intronic promoter of the short ACE2 isoform in renal proximal tubules." *bioRxiv: the preprint server for biology* 2021.01.15.426908. 19 Jan. 2021, doi:10.1101/2021.01.15.426908. Preprint.

Kyritsis, Nikos et al. "Acute Inflammation Initiates the Regenerative Response in the Adult Zebrafish Brain." *Science* 338 (6112), 1353-1356. DOI: 10.1126/science.1228773 originally published online November 8, 2012

Lafreniere, Denis. "Taste and Olfactory Disorders in Adults: Evaluation and Management." *UpToDate*, May 2022, https://www.uptodate.com/contents/taste-and-olfactory-disorders-in-adults-evaluation-and-management#!

Liang, Fengyi. 2020. "Sustentacular Cell Enwrapment of Olfactory Receptor Neuronal Dendrites: An Update" *Genes* 11, no. 5: 493. https://doi.org/10.3390/genes11050493

L. D’Ascanio, F. Vitelli, C. Cingolani, M. Maranzano, M.J. Brenner, A. Di Stadio. Randomized clinical trial "olfactory dysfunction after COVID-19: olfactory rehabilitation therapy vs. intervention treatment with Palmitoylethanolamide and Luteolin": preliminary results. *Eur Rev Med Pharmacol Sci*. 2021 Jun;25(11):4156-4162. doi: 10.26355/eurrev_202106_26059. PMID: 34156697.

Parker, J.K., Kelly, C.E. & Gane, S.B. Insights into the molecular triggers of parosmia based on gas chromatography olfactometry. *Commun Med* 2, 58 (2022). https://doi.org/10.1038/s43856-022-00112-9

D'Ascanio L, Vitelli F, Cingolani C, Maranzano M, Brenner MJ, Di Stadio A. Randomized clinical trial "olfactory dysfunction after COVID-19: olfactory rehabilitation therapy vs. intervention treatment with Palmitoylethanolamide and Luteolin": preliminary results. *Eur Rev Med Pharmacol Sci*. 2021 Jun;25(11):4156-4162. doi: 10.26355/eurrev_202106_26059. PMID: 34156697.

Roskams, A.J.; Cai, X.; Ronnet, G.V. Expression of neuron-specific beta-III tubulin during olfactory neurogenesis in the embryonic and adult rat. *Neuroscience* 1998, 83, 191–200. [Google Scholar] [CrossRef]

Iqbal T., Byrd-Jacobs C. Rapid degeneration and regeneration of the zebrafish olfactory epithelium after triton X-100 application. *Chem. Senses*. 2010;35:351–361. doi: 10.1093/chemse/bjq019.

Scheib, Jackson, and Christine Byrd-Jacobs. “Zebrafish Astroglial Morphology in the Olfactory Bulb Is Altered With Repetitive Peripheral Damage.” *Frontiers in neuroanatomy* vol. 14 4. 11 Feb. 2020, doi:10.3389/fnana.2020.00004

Thompson-Souza GA, Gropillo I, Neves JS. Cysteinyl Leukotrienes in Eosinophil Biology: Functional Roles and Therapeutic Perspectives in Eosinophilic Disorders. *Front Med* (Lausanne). 2017;4:106.