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Amperometric Detection of Neurotransmitter Release from Taste Buds in Response to Sour and Fatty Tastants

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Abstract

The goal of this project is to elucidate the mechanism by which taste signals are transduced within taste buds using an electrochemical technique known as amperometry. The sense of taste is important for animals because it allows animals to recognize food, derive pleasure from food, and to detect food that may be unsafe to eat. The neurotransmitters serotonin and norepinephrine are released from taste cells in response to taste stimuli. Amperometry has been used to detect the release of these neurotransmitters from taste buds. This technique has been used to identify and characterize the taste responses to sour and fatty tastants.

Introduction to Amperometry

•Amperometry can be used to detect release of electrically active compounds

•Electrochemically active neurotransmitters, like serotonin, can be detected through oxidation reactions carried out at the surface of the electrode

•Set potential to +0.8 V to oxidize serotonin

•Oxidation is detected as change in current

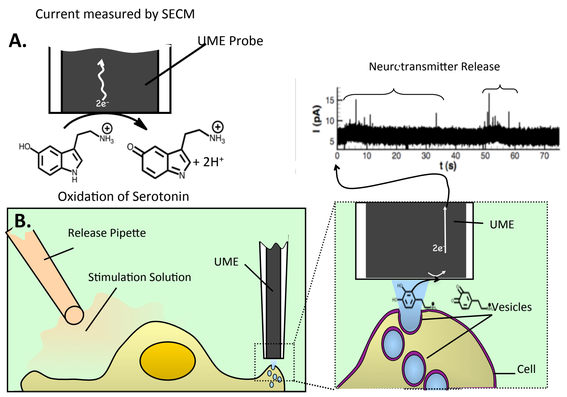


Figure 1.

Schematic **A** represents of the oxidation of serotonin by the electrode.

Cartoon **B** shows the experimental set up and an example of the amperometry data.

Multiple Fiber Carbon Electrodes

•Multiple, separate carbon fibers

•Larger surface area of electrode

•Increases probability of seeing neurotransmitter release

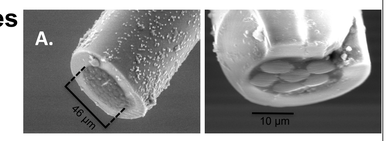
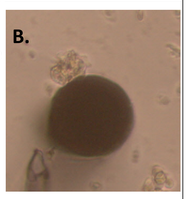


Figure 2.

Figure **A** SEM image multiple fiber carbon electrode.

Figure **B** shows a multiple carbon fiber electrode in solution with taste buds.



Acknowledgments

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Illinois Wesleyan University, Chemistry Department
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References

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Taste Bud Background

•There are five kinds of taste: salty, bitter, sour, sweet, fat and umami

•Taste buds are a group of 50-100 cells and are responsible for initiating sensation of taste

•Taste buds are made up of at least three different kinds of cells: Type I (Glial-Like), Type II (Receptor Cells) and Type III (Pre-Synaptic Cells).

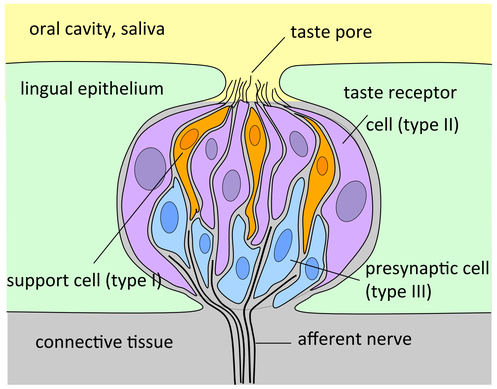

Figure 3.
Cartoon of taste bud containing the three different cell types

Schematic for Taste Signaling Mechanism

•Type II cells transduce umami, sweet, and bitter tastants through receptor proteins.[1]

•After the tastant is transduced in Type II cells an ATP channel is opened, releasing ATP which is detected by type III cells.

•In addition to signaling Type III cells also transduce sour tastants, this mechanism is shown in Figure 4B.

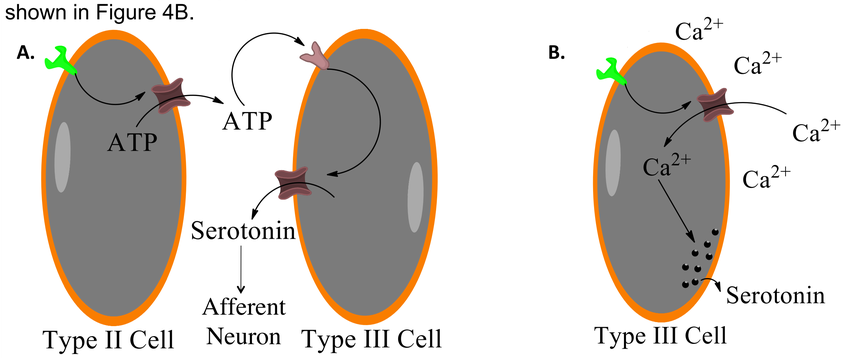

Figure 4.
A. Signal transduction in receptor (Type II) cells involves opening an ATP channel. Extracellular ATP actives a GCPR response on the receptor cell and results in the release of serotonin from pre-synaptic (Type III) cells.
B. Stimulation of Type III cells through acidic or KCl stimulation opens an extracellular calcium channel. The increase in intracellular calcium concentration causes serotonin to be released.

Results

Experiments are conducted by stimulating taste buds with different classes of tastants while monitoring a taste bud for neurotransmitter release. After successful detection of neurotransmitter from a taste bud, experimental conditions were altered to by either stimulating in a calcium free environment or adding an ATP channel blocker, suramin, to solution. If neurotransmitter release of a class of tastants is dependent upon the presence of extracellular calcium this indicates that the tastant directly stimulates type III cells. Conversely, if neurotransmitter release of a class of tastants is dependent on the lack of the ATP channel blocker this indicates that the tastant is transduced by type II cells.

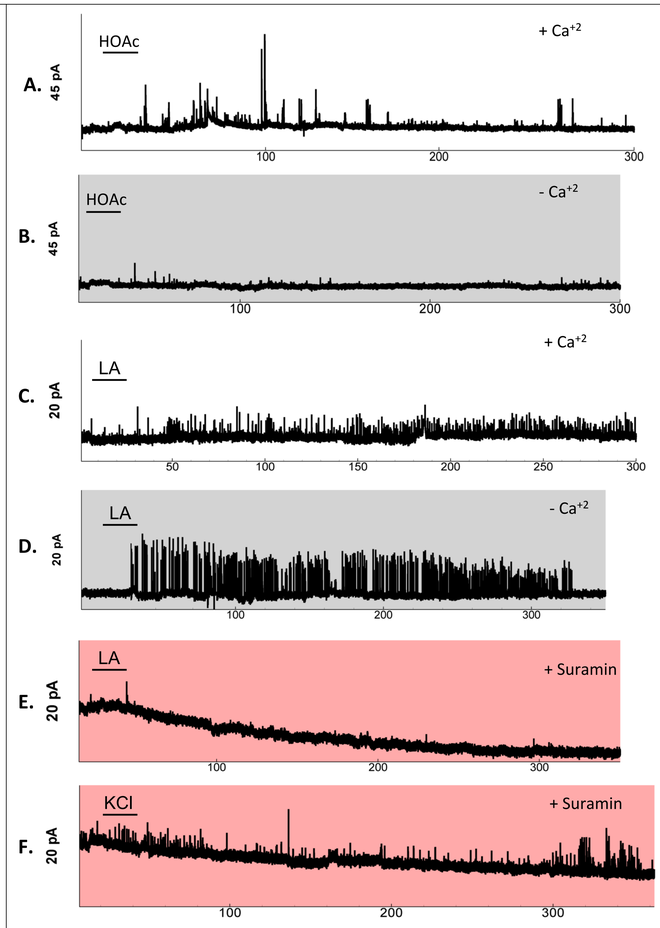

Figure 5.
A. and **B.** Amperometric data when taste buds stimulated with acetic acid in the presence of extracellular calcium and in a calcium free solution
C. and **D.** Amperometric data when taste bud was stimulated using linoleic acid (LA) with and without extracellular Ca^{2+}.
Figure **E.** Stimulation with linoleic acid in the presence of ATP channel blocker suramin
Figure **F.** shows same taste bud as Figure C and D being stimulated with KCl in the presence of suramin.

Conclusion

The stimulation with acidic acid (shown in Figure 5A and B) shows a clear dependence on the extracellular calcium concentration, indicating that as previously believed sour tastants directly stimulate type III cells. Similar experiments with linoleic acid (LA) did not show this dependence on extracellular calcium (shown in Figure 5 C and D). However, as shown in Figure 5E, neurotransmitter release upon stimulation with LA was dependent on the lack of an ATP channel inhibitor. Figure 5F shows the same taste bud releasing neurotransmitter while being stimulated with KCl (transduced like acetic acid) with the ATP channel blocker present. This suggests fatty acid tastants are transduced similar to bitter, sweet and unami tastants through transduction by type II cells which then use ATP to communicate with type III cells.

Future Work

In the future we wish to use GFP labeled cells and fluorescence microscopy to determine each cell type's role in transduction using amperometry. Also we wish to topographically image cells during stimulation