Long term *in vivo* one-photon Ca^{2+} imaging of taste-responsive cells in the parabrachial pons in the awake freely licking rat.

BINGHAMTON UNIVERSITY
State University of New York

O'Connell, FP[1]; Sammons, JD[2]; Di Lorenzo[1], PM

oconnell@Binghamton.edu; jsammons@uab.edu; diloren@Binghamton.edu

[1]Dept. of Psychology, Binghamton University Binghamton, NY; [2]Current address: Dept. of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL

I. INTRODUCTION

- The observation that cells at all levels of the taste pathway respond primarily to a single taste quality, constant over time, is the basis of **labeled line** theory. However, studies have shown that only a subset of cells in each neural structure are narrowly tuned. Further, tuning changes over time have been observed.
- The **parabrachial nucleus of the pons (PbN)** receives input from the nucleus of the solitary tract and ultimately projects to the insular cortex through the ventromedial thalamus.
  - Recent evidence suggests that taste-responsive cells in the mouse PbN can be either narrowly or broadly tuned across taste qualities.[1]
  - Also the same properties are shown by neurons in the gustatory cortex[2].
- We used one-photon *in vivo* Ca^{2+} imaging to track the activity of a population of PbN cells over days/weeks.
- We tested the **hypothesis** that the tuning properties of PbN taste cells would change over time but firing patterns across the population would remain informative.

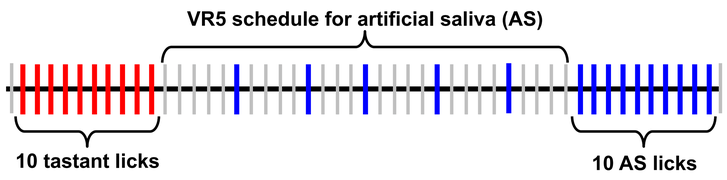
II. METHODS

Rats (n= 3) were prepared for Ca^{2+} imaging:

- The PbN (11.3 A/P, 1.7 M/L, -5.6 D/V) was infused with AAV9 virus containing the GCaMP7s gene under the synapsin 1 promoter (Addgene #104487-AAV9; pGP-AAV-syn-jGCaMP7s-WPRE) GRIN lens into PbN (same coordinates)

Following recovery, rats were water deprived and a miniscope (Inscopix, Inc.) was mounted to the surgical baseplate.

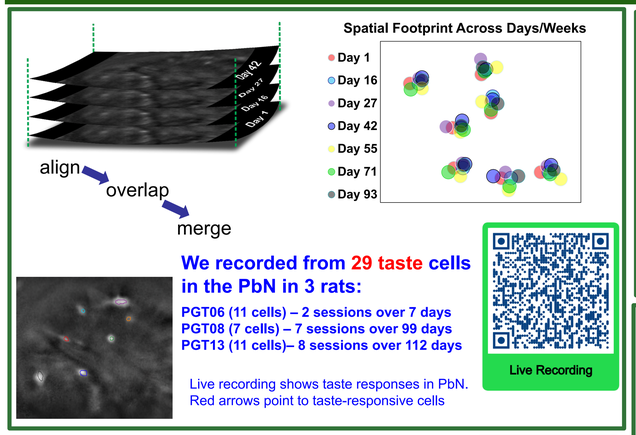
- Rats were placed in an operant chamber with free access to taste stimuli representing the 5 basic taste qualities.
- Tastants were dissolved in artificial saliva (AS) and included:
  - **Sucrose** (0.1 M), **NaCl** (0.1 M), **Citric Acid** (0.016 M), **Quinine** (0.0001 M), **MSG / IMP** (0.1 M / 0.01 M) and **AS**.

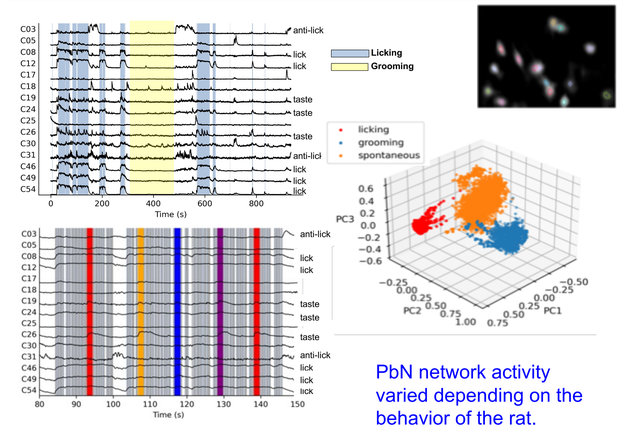


Data analysis:

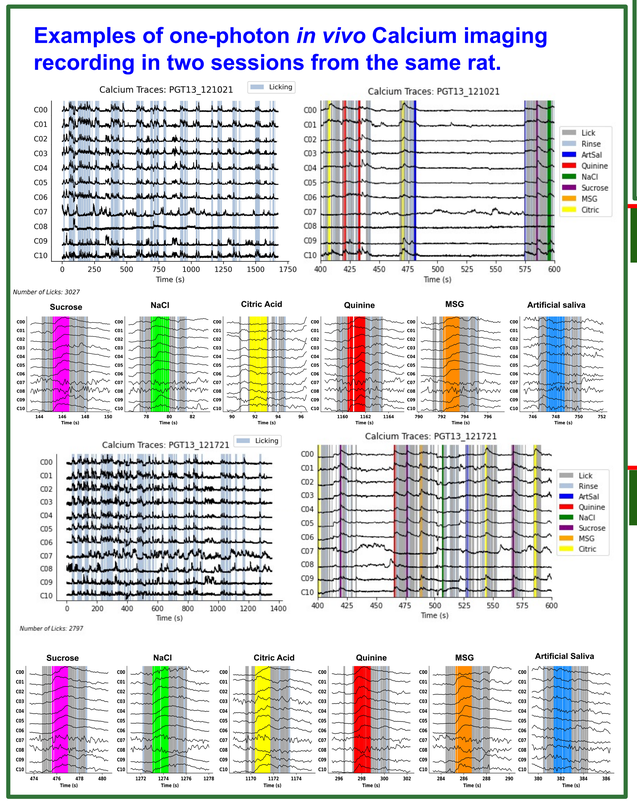
- Video acquisition with nVista® software (Inscopix, Inc.) at a sample rate of 10 Hz with a single focal plane representing the sharpest image.
- Video processing with Inscopix Data Processing software (Inscopix DPS®, Inc.) in several steps:
  - Spatial downsampling (4x), bandpass filter, motion correction, manual ROI extraction, contour application
- Final output was $\Delta F/F_0$ where F_0 where represents *mean* fluorescence across the recording period.
- Taste responses were calculated as the average signal + 2.58SD above a 4s pre-stimulus baseline

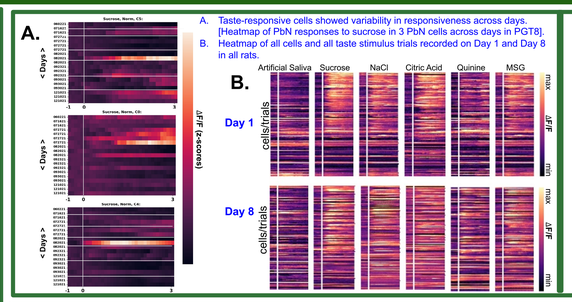
III. RESULTS

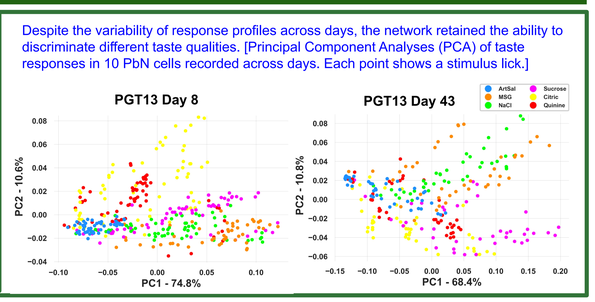




Taste-responsive cells were recorded alongside anti-lick and lick bout cells, as in electrophysiological experiments (see Roussin et al. 2012).



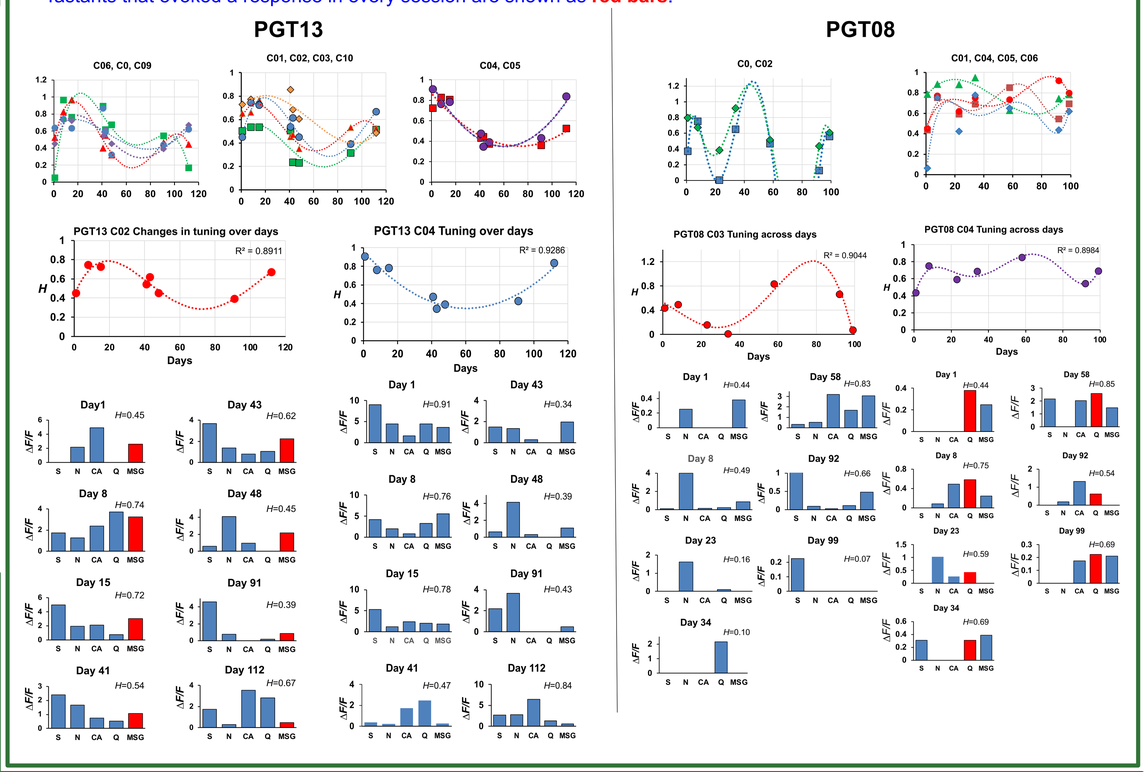




Tuning of PbN cells across days/weeks. Breadth of tuning was assessed using the Entropy measure (Smith & Travers, 1979) as follows: $H = -1.661 \sum_{n=1}^{4} P_i(\log P_i)$

Many cells showed systematic variations in tuning across days/weeks.

Tastants that evoked a response in every session are shown as **red bars**.



IV. Conclusions

- ★ Taste responses in the PbN of awake, freely licking rats were recorded using one-photon Ca^{2+} imaging.
- ★ 29 taste-responsive PbN cells were recorded from three rats over several sessions spanning several weeks. Lick bout and anti-lick cells were also recorded.
- ★ Variability in tuning of taste-responsive cells across days was ***systematic*** in some cells but relatively stable in others. Often, a cell's response to one or more tastants was always present across days (shown in **red** bars), though it wasn't necessarily the "best" stimulus. Thus, "***labeled lines***" may be defined by the tastant that evokes the most consistent response over time.

V. Acknowledgements

Funded by NIDCD Grant RO1-DC006914 to PMD.

We are grateful to Dr. Morgana Favero and Dr. Jing Liang-Guallpa for their advice and support.

NIDCD

References

1. Chen K, Kogan JF, Fontanini A. Spatially Distributed Representation of Taste Quality in the Gustatory Insular Cortex of Behaving Mice. *Curr. Biol.*, **31(2):** 450 (2021).
2. Jarvie BC, Chen JY, King HO, Palmiter RD. Satb2 neurons in the parabrachial nucleus mediate taste perception. *Nat Commun* **12:** 224 (2021).
3. Roussin AT, D'Agostino AE, Fooden AM, Victor JD, Di Lorenzo PM. Taste coding in the nucleus of the solitary tract of the awake, freely licking rat. *J Neurosci.* **32(31)**: 10494-506 (2012)
3. Smith DV, Travers, JB. A metric for the breadth of tuning of gustatory neurons. *Chem Senses Flav.* **4**: 215-229 (1979).
4. Yarmolinsky DA, Zuker CS, Ryba NJP. Common sense about taste: from mammals to insects. *Cell* **139**: 234–244 (2009).