Neural population responses to taste and food in the parabrachial nucleus of the pons in the awake unrestrained rat using *in vivo* one-photon Ca^{2+} imaging

Binghamton University, State University of New York

Flynn P. O'Connell[1], Jonathan D. Victor[2], Patricia M. Di Lorenzo[1]

oconnell@Binghamton.edu; jdvicto@med.cornell.edu; diloren@Binghamton.edu

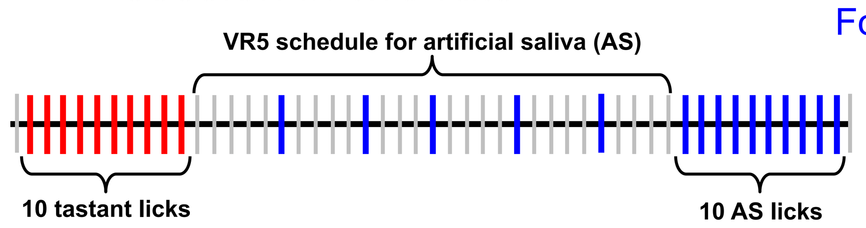
[1]Dept. of Psychology, Binghamton University Binghamton, NY; [2]Feil Family Brain and Mind Research Institute, Weill Cornell Medical College, New York, New York 10065.

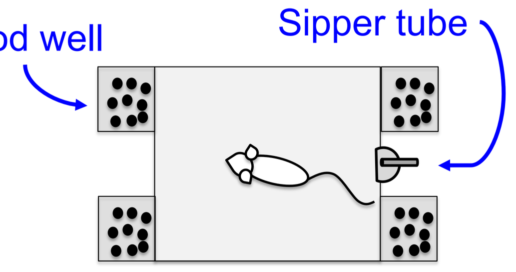
I. INTRODUCTION

- Although brainstem responses to taste stimuli have been well-documented, the function of the taste system in natural feeding remains unexplored.
- Electrophysiological data from the nucleus of the solitary tract (NTS) suggested that the taste system is strongly engaged while exploring food and sampling its taste and smell but becomes relatively disengaged when food is being consumed.
- The **parabrachial nucleus of the pons (PbN)** receives direct input from the NTS and ultimately projects to the insular cortex through the ventromedial thalamus.
- We used one-photon *in vivo* Ca^{2+} imaging to track the activity of a population of PbN cells as they licked tastants, smelled food and ate solid food.
- We tested the **hypothesis** that PbN cells inherit key properties from NTS. In particular that:
  - Like NTS, PbN neurons would respond not only to the taste of food but also to its smell
  - Like NTS, PbN neurons would respond strongly while exploring and sampling, but relatively quiescent during eating and consumption

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). Two weeks later, a GRIN lens was implanted with the tip positioned just above the PbN.
- Following recovery, rats were water deprived and a miniscope (Inscopix, Inc.) was mounted to the surgical baseplate.
- Rats were first placed in an operant chamber with free access to taste stimuli representing basic taste qualities. Two olfactory stimuli were also presented along with artificial saliva (AS): peanut or chocolate odor.
- Tastants were dissolved in artificial saliva (AS) and included:
  - 0.1 M sucrose, 0.1 NaCl, 0.0167 M citric acid, 0.0001 M quinine) as well as AS presented alone or paired with either peanut or chocolate odor.
  - Solid foods (Granny Smith apple, salted peanuts or milk chocolate were available in food wells after the licking session in two rats.



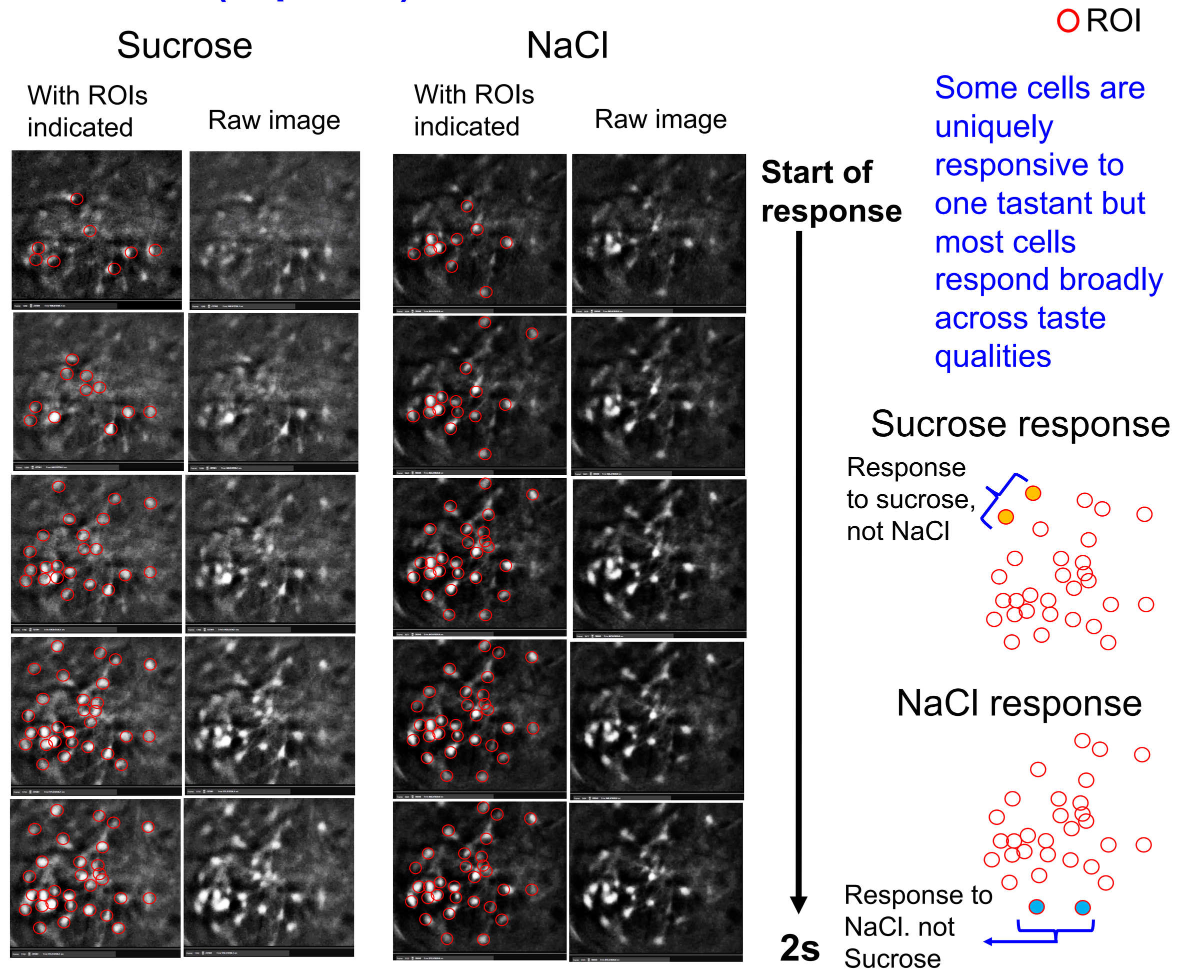


Data analysis:

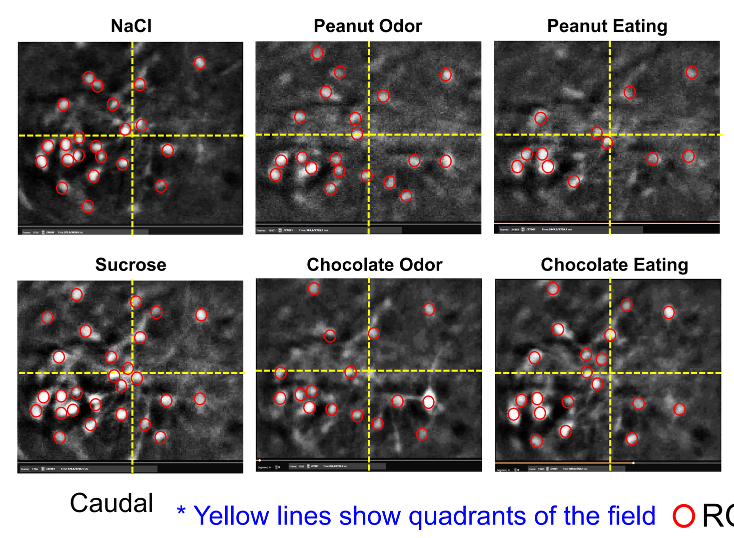
- Video acquisition was done with nVista® software (Inscopix, Inc.) at a sample rate of 10 Hz
  - A single focal plane representing the sharpest image was selected.
- Video processing with Inscopix Data Processing software (Inscopix DPS®, Inc.) in several steps:
  - Spatial downsampling (4x), bandpass 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.

III. RESULTS

The spatial response to taste in the PbN evolves (expands) over time PGT13



Some cells are uniquely responsive to one tastant but most cells respond broadly across taste qualities

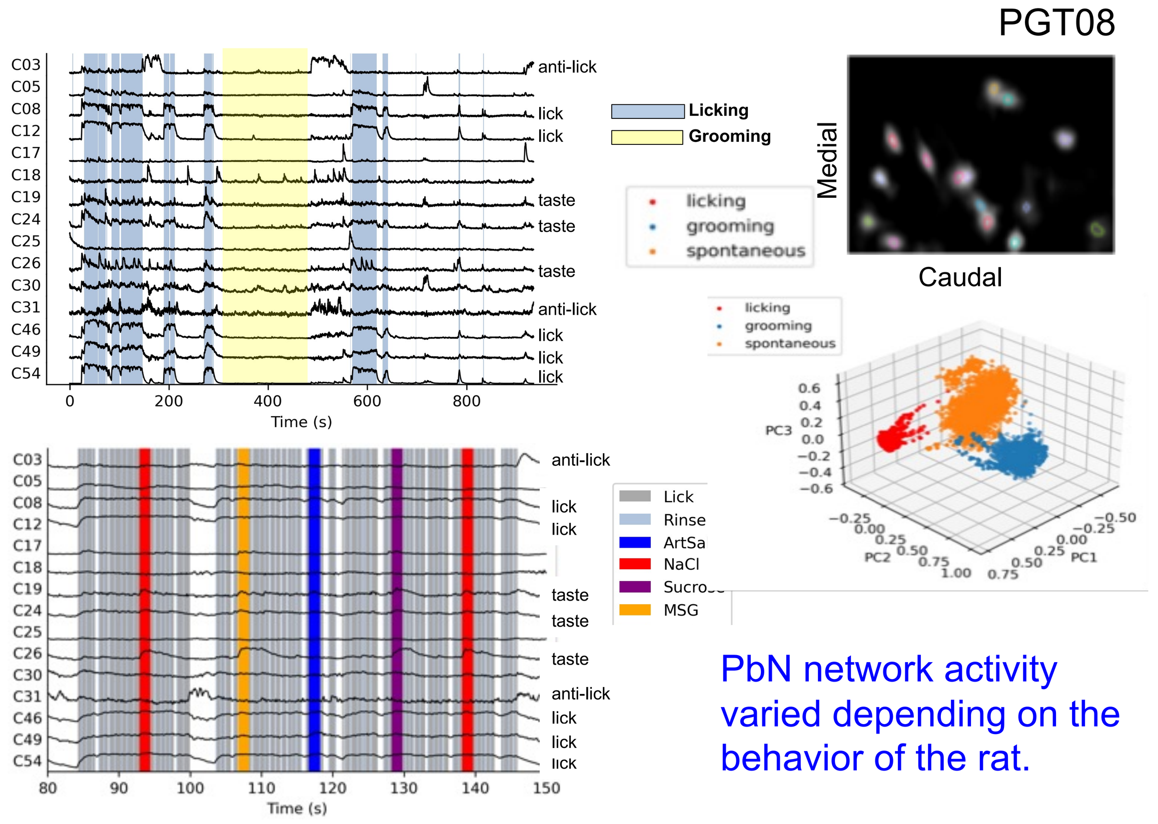


* Yellow lines show quadrants of the field ROI

When rats ate either peanuts or chocolate, responsive cells were predominantly located in a small cluster located in the caudo-medial field

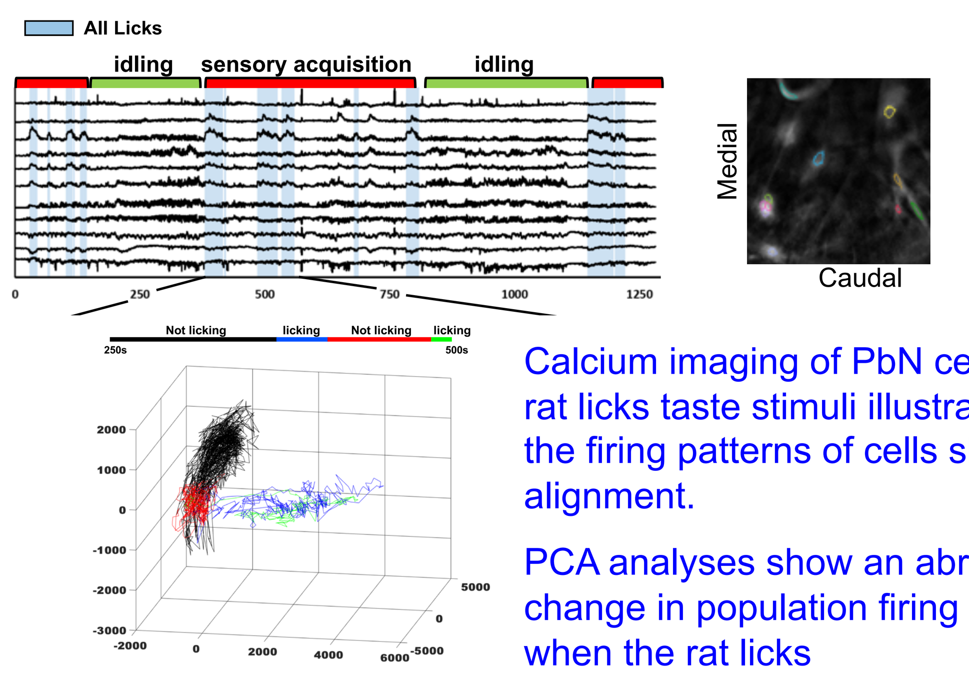
There was no gustotopic segregation of tastants

PGT08



PbN network activity varied depending on the behavior of the rat.

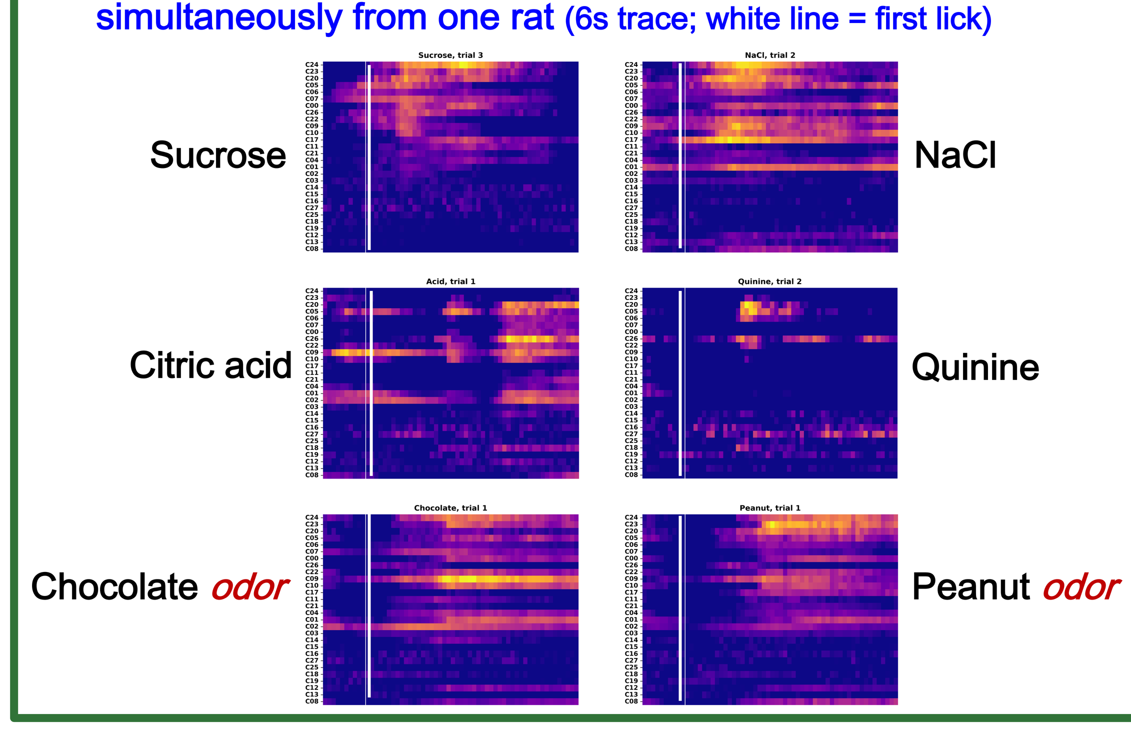
Taste-responsive cells were recorded alongside anti-lick and lick bout cells, as in electrophysiological experiments (see Roussin et al. 2012).



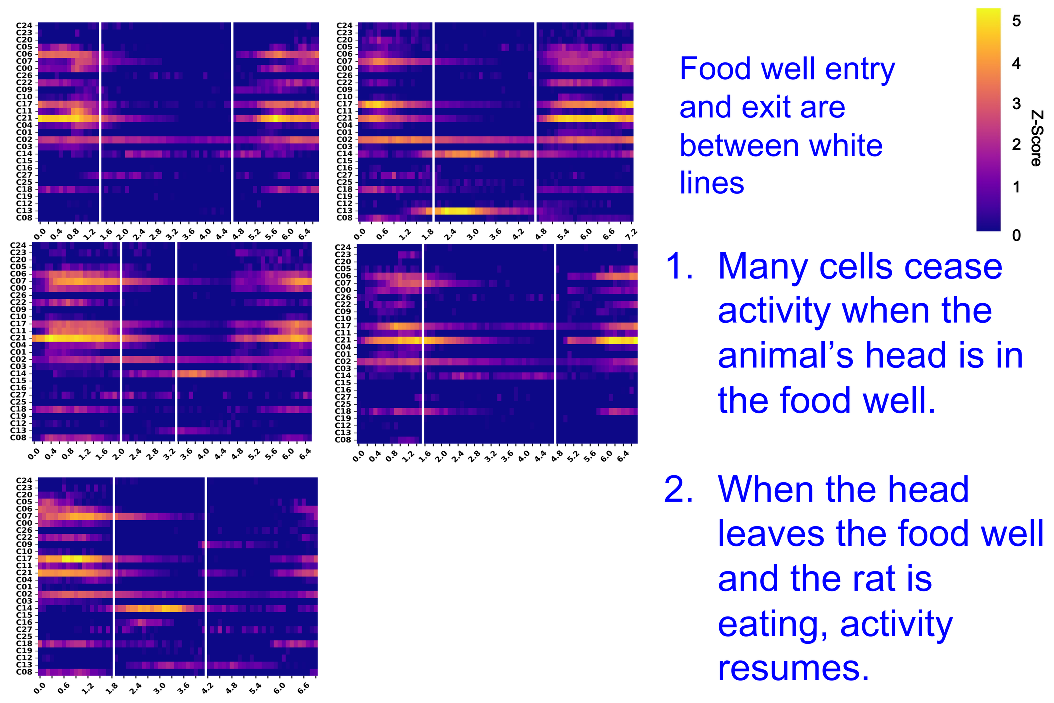
Calcium imaging of PbN cells as the rat licks taste stimuli illustrates that the firing patterns of cells shift in alignment.

PCA analyses show an abrupt change in population firing patterns when the rat licks

Heatmaps of responses in 28 PbN cells recorded simultaneously from one rat (6s trace; white line = first lick)



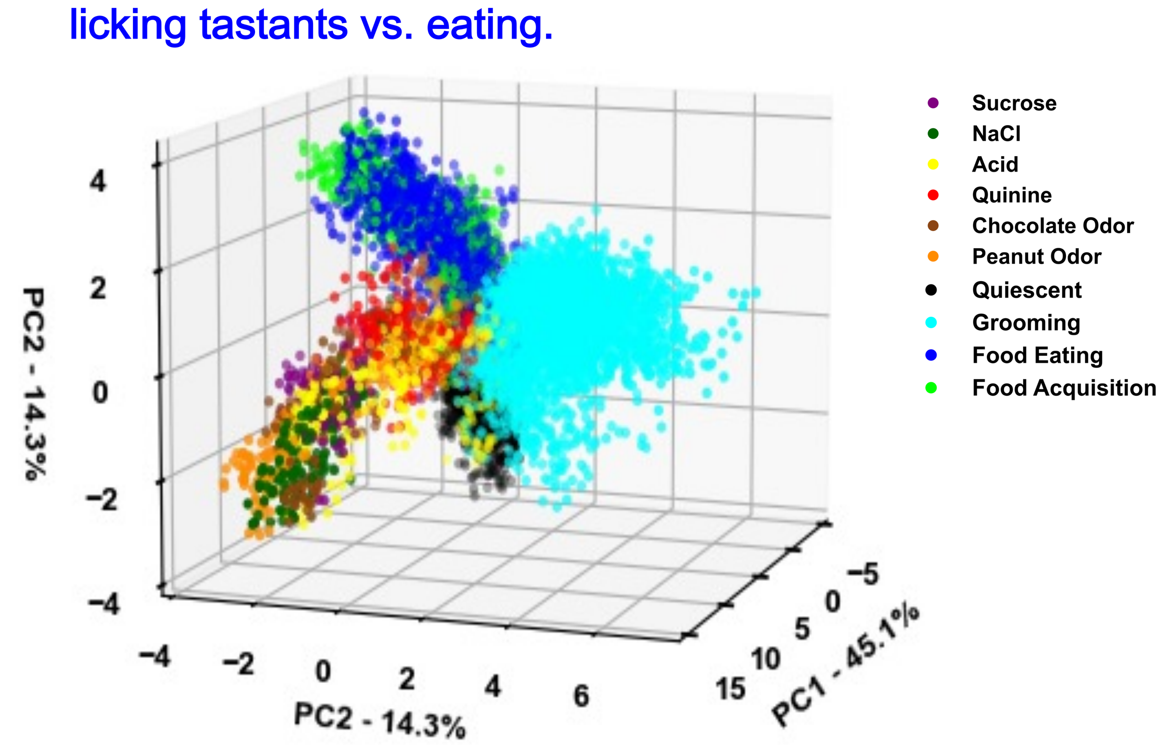
Peanut food well entry, food well exit and eating PGT13

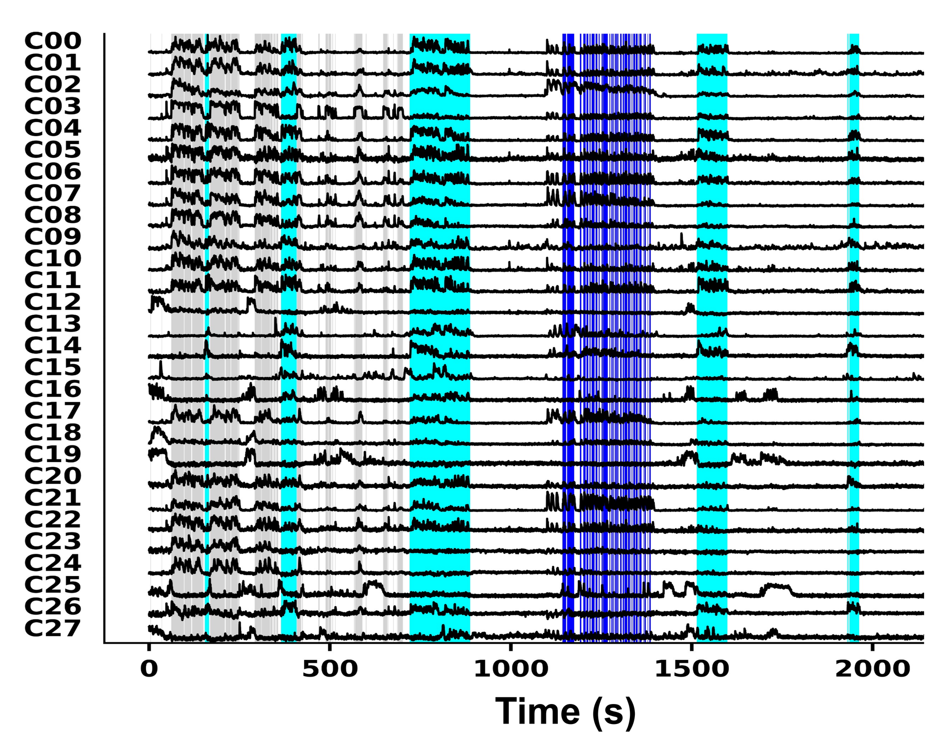


Food well entry and exit are between white lines

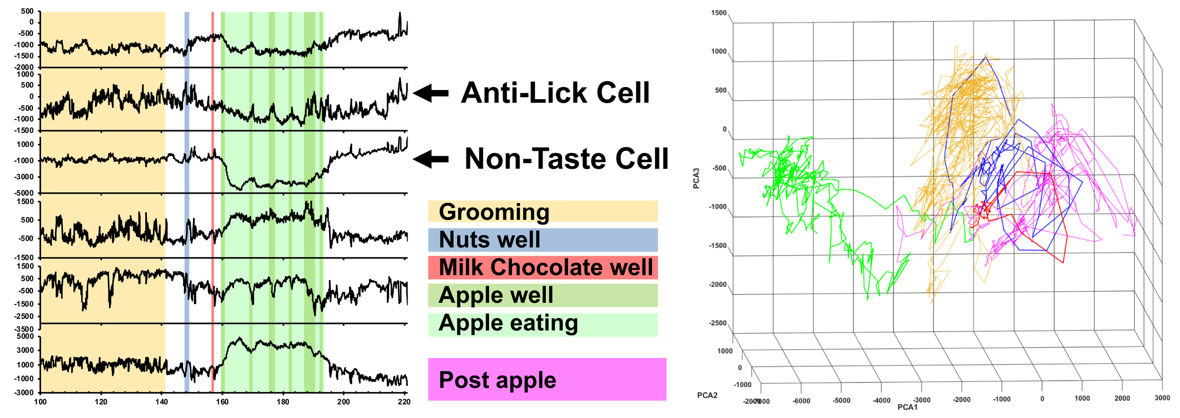
1. Many cells cease activity when the animal's head is in the food well.
2. When the head leaves the food well and the rat is eating, activity resumes.

Network activity is different when the rat is licking tastants vs. eating. PGT13





PGT08



When a rat engages in appetitive and consummatory behavior, ***all PbN cells*** change their mode of firing in an organized way

IV. Conclusions

- Taste responses in the PbN of awake, freely licking rats were recorded using one-photon Ca^{2+} imaging. Taste-responsive PbN cells were recorded from three rats. Records were obtained from two rats while they ate solid food.
- **Taste responses evolved over space and time without a clear gustotopic organization**
- In one rat (PGT13) records were obtained from 28 PbN cells as the rat licked tastants, smelled chocolate or peanut odor and ate chocolate and peanuts. **Results clearly indicate that PbN cells respond to odor as well as taste.**
- **PbN network activity changes abruptly when a rat licks or eats.** Activity during appetitive and consummatory behavior is likely ***multimodal***, reflecting taste, olfactory and motor-related signals.

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.

References

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)