Differences in Retro- and Orthonasal Olfaction in Rat Chemosensory Cortices

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Introduction & Background

Optogenetic Inhibition of Gustatory Cortex Influences Piriform Cortex

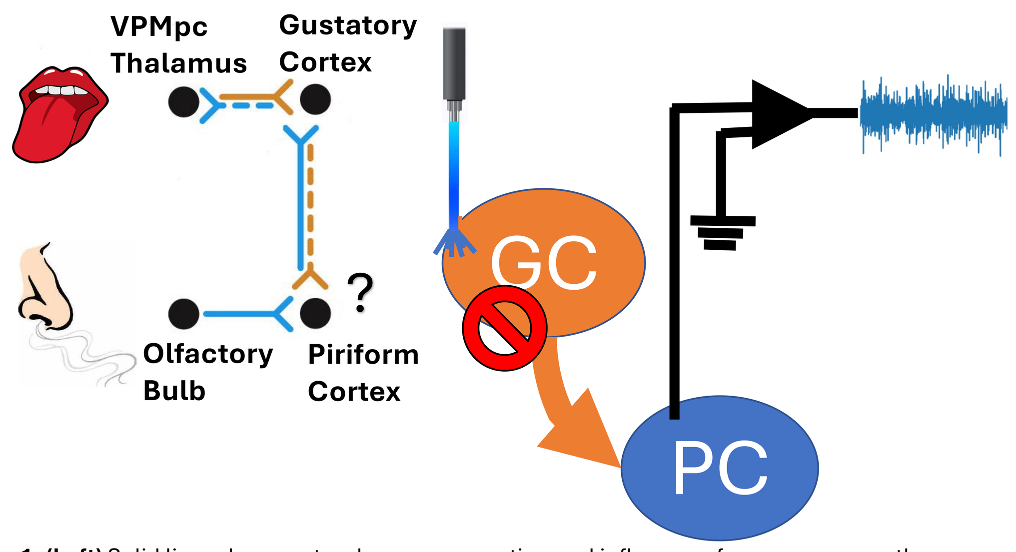


Figure 1. (Left) Solid lines demarcate a known connection and influence of one area on another. Dotted lines indicate an uncharacterized but predicted connection. **(Right)** Functional connectivity diagram of gustatory cortex (GC) and piriform cortex (PC) describing a previous experiment[1] where GC excitatory neurons were inhibited with optogenetics and PC neurons were recorded from with single channel electrodes.

Orthonasal and Retronasal Olfaction in Mammals

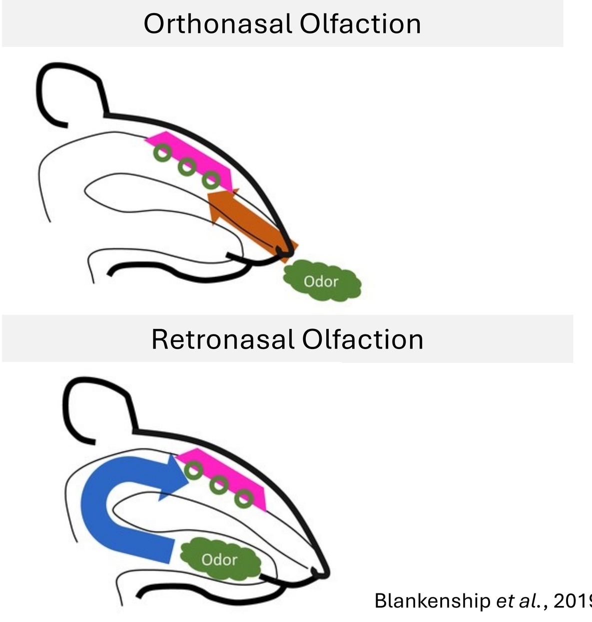
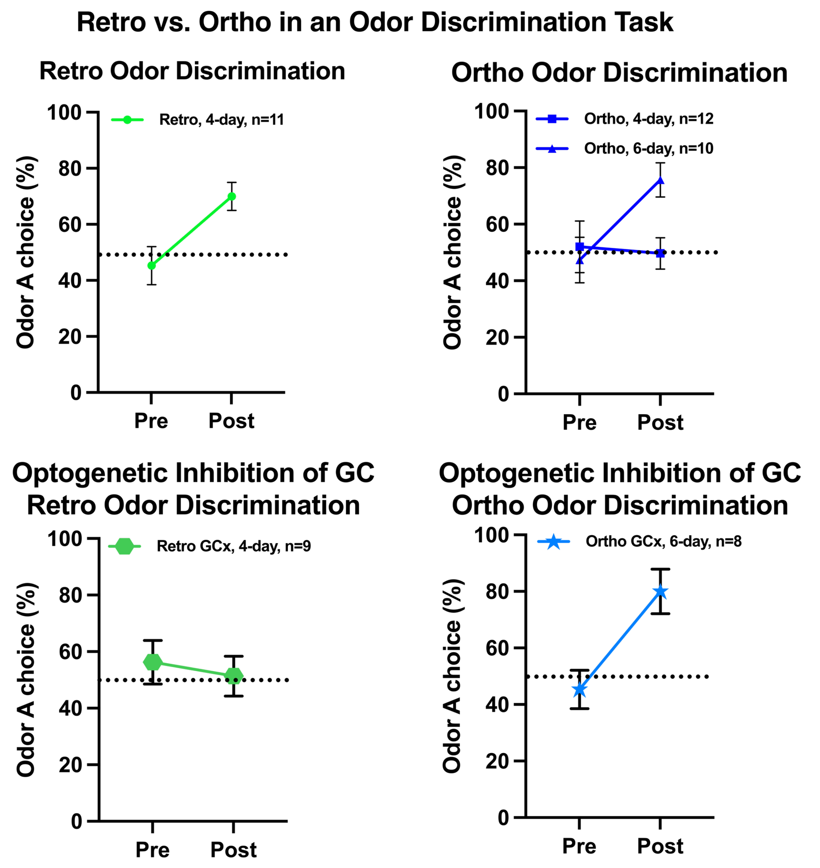


Figure 2. (Top) Visual depiction of orthonasal olfaction in rats. **(Bottom)** Visual depiction of retronasal olfaction in rats.[2]

Optogenetic Inhibition of Gustatory Cortex Influences Piriform Cortex



Optogenetically inhibiting GC changes baseline activity in PC and changes odor responses

Retronasal acquisition of odor discrimination learning happens faster than orthonasal

Optogenetically manipulating GC demonstrates that GC is required for retronasal but not orthonasal olfactory function.

Figure 3. (Top Left) Odor discrimination task using retronasally delivered odors. "Pre" training odor choice assessment followed by reward training for 4 days with Odor A and a "Post" training odor choice assessment. The dotted line represents a 50% of choosing Odor A over B (chance). **(Top Right)** Ortho odor discrimination task with 4 days of training and with 6 days of training. **(Bottom Left)** The same paradigm as above with optogenetic inhibition of GC. **(Bottom Right)** The same paradigm with optogenetic inhibition of GC.

Adapted from Blankenship et al., 2019

Objectives

Specific Aim: Characterize the dynamic activity between Gustatory Cortex and Piriform Cortex in response to retronasal olfaction and orthonasal olfaction and identify whether there is phase coherence.

1. Determine where in the odor response are LFP signals significantly coherent between GC and PC.
2. See whether GC and PC neuron pairs respond more coherently with orthonasal olfaction than retronasal olfaction.
3. Get insight on any directionality of cortical responses between GC and PC.

Approach

Experimental Protocol for Retronasal Delivery

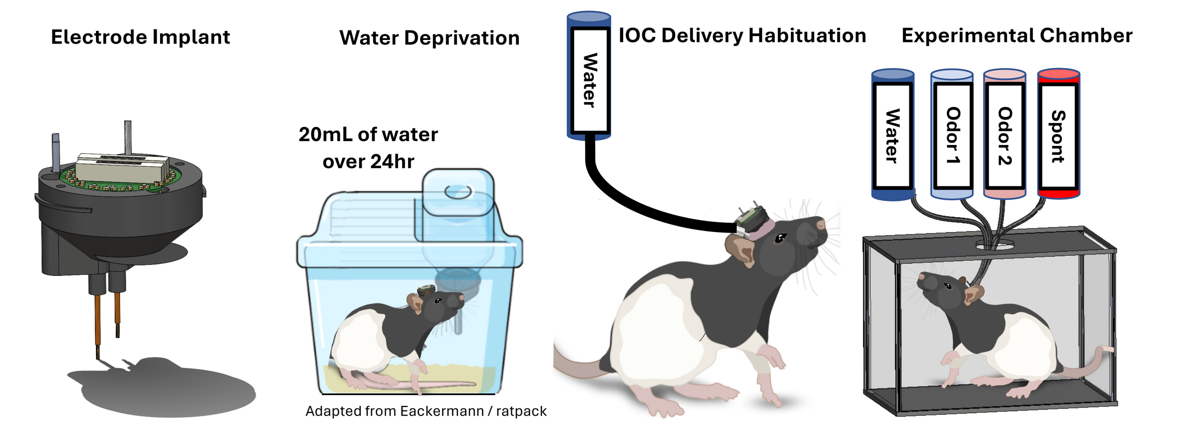
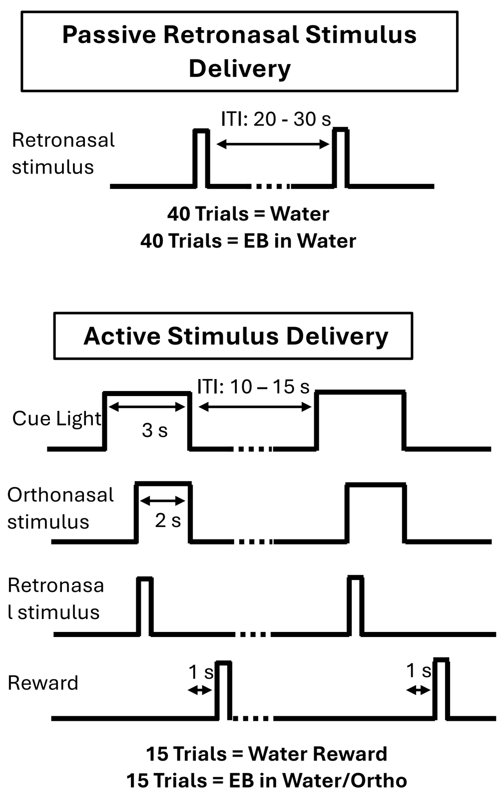


Figure 5. (Left) Dual site drivable electrode array. 6 days after surgery rats are water deprived (given 20mL for 24 hours). On days 7 & 8, rats are given water through an intraoral cannula (IOC). **(Right)** On days 9-11, rats are given 0.01% Ethyl Butyrate dissolved in water, and water. Spontaneous activity (Spont) is also recorded as a control at the beginning of the experiment.



Adapted from Maier et al., 2015

Experimental Protocol for Orthonasal Delivery

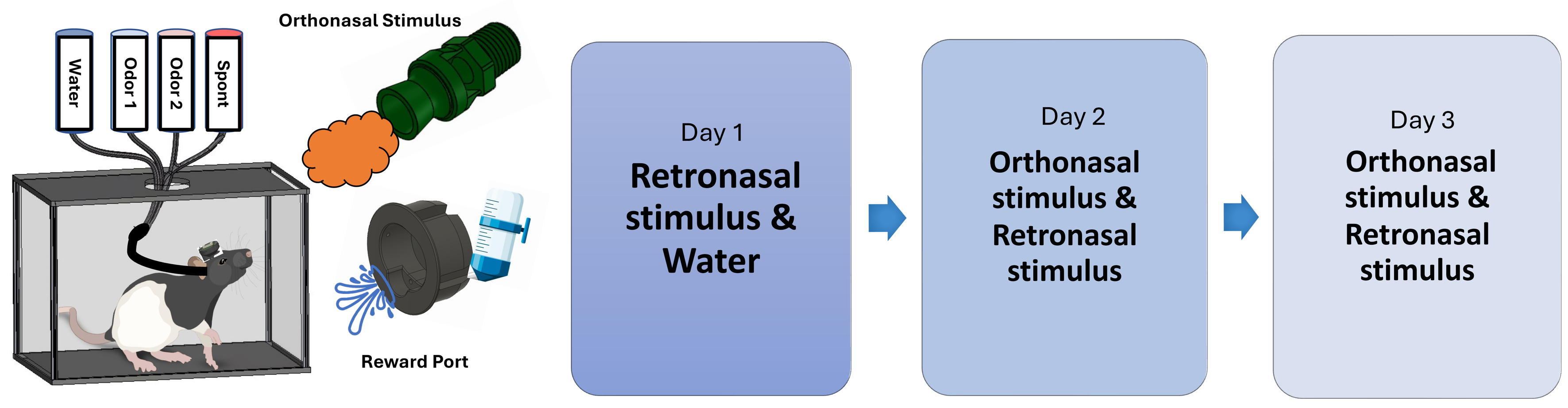
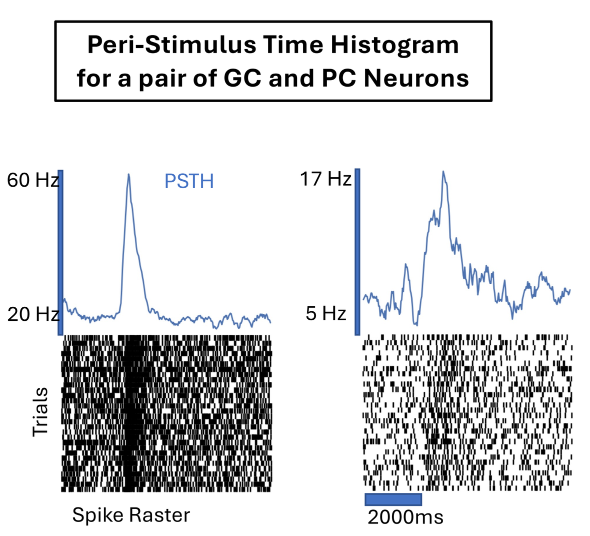


Figure 6. (Left) Stimulus delivery protocol. Cue light signals water is available, then either a retro or ortho stimulus is delivered, and finally a water reward is delivered through the reward port. **(Middle)** Rat connected to IOC for retronasal odor deliveries. Orthonasal stimulus delivered via olfactometer[3] and IR beam triggered reward port. **(Right)** Experimental protocol for active acquisition of ortho- and retronasal stimuli.

PSTH and Phase Coherence Analyses

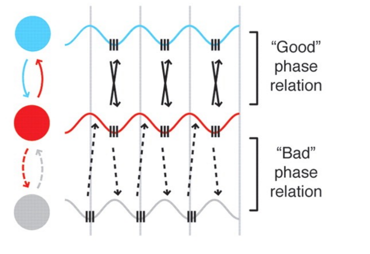


Analysis Methods

- Peri-Stimulus Time Histograms
- LFP Phase Coherence of GC and PC
- Trial Shuffled Comparisons of Neuron Pairs
- Changepoint Model of State Transitions during the odor response
- Grainger Causality of State Transitions

$$Coherence_{GC\leftrightarrow PC}(f) = \left|\frac{1}{N}\sum_{t=1}^{T} e^{-i(\theta_t^{GC}(f)-\theta_t^{PC}(f))}\right|$$

Equation 1. Phase coherence analysis can be performed using this , to evaluate the coupling between GC and PC LFPs during retronasal olfaction[4].



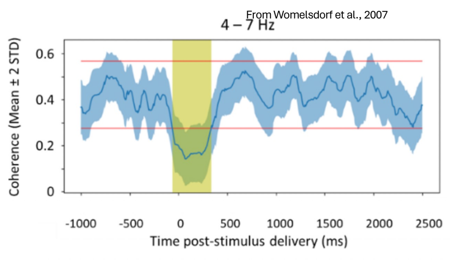


Figure 7. (Left) Example of Peri-stimulus time histograms with corresponding spike raster below. **(Right Top)** Phase relation diagram demonstrating phase coherence. **(Right Bottom)** Example phase coherence analysis of GC and the Basolateral Amygdala during passive taste delivery. The blue is the phase coherence over time and the yellow is a significant deviation from baseline.

Results

Peri-Stimulus Time Histograms for GC and PC during Ortho- Retronasal Olfaction

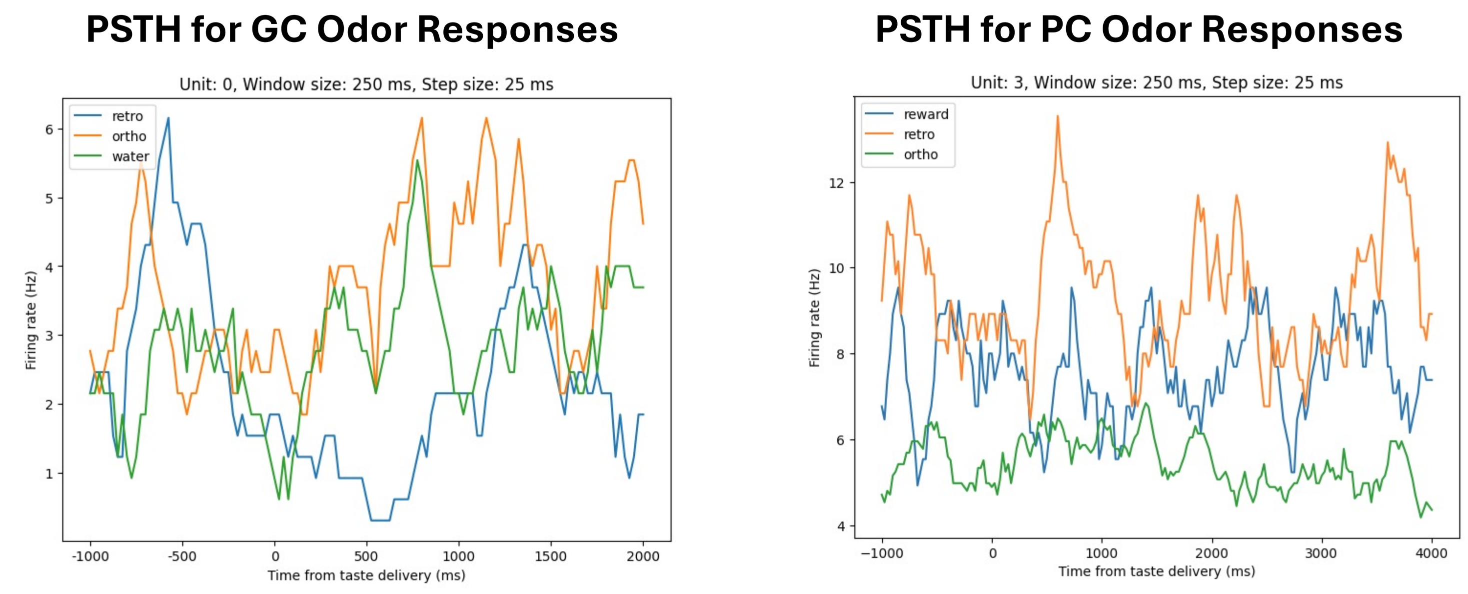
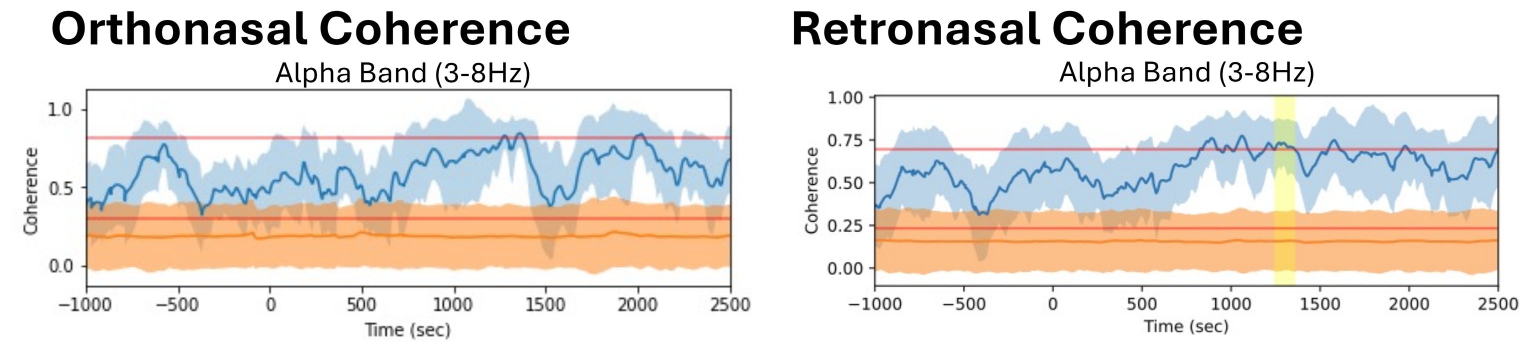
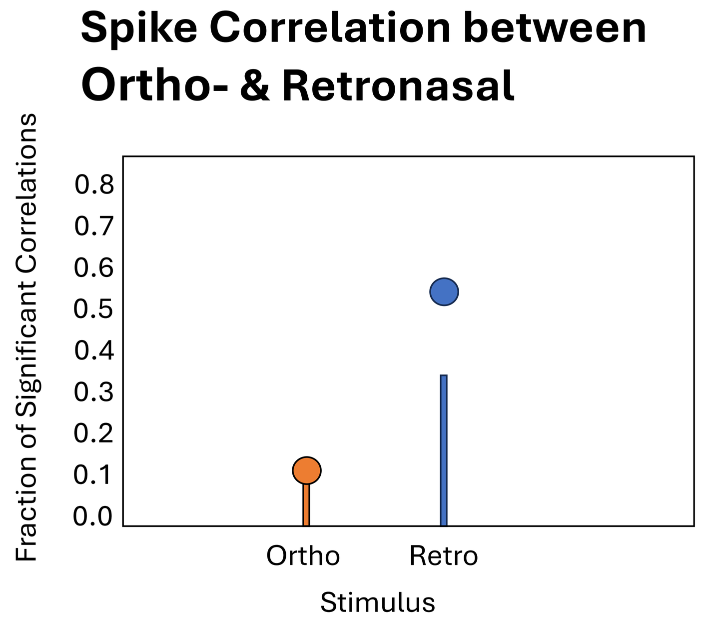


Figure 8. (Top Left). Peri-stimulus time histogram showing GC representative neuron responses in hertz to retronasally delivered 0.01% ethyl butyrate in water, water, and orthonasally delivered ethyl butyrate at 0.01% concentration in mineral oil. Each stimulus was delivered 15 times. **(Top Right)** PSTH for PC response to to the same battery of stimuli as GC. These two examples were recorded in the same animal.

Coherence between GC and PC LFPs during Ortho- and Retronasal Olfaction





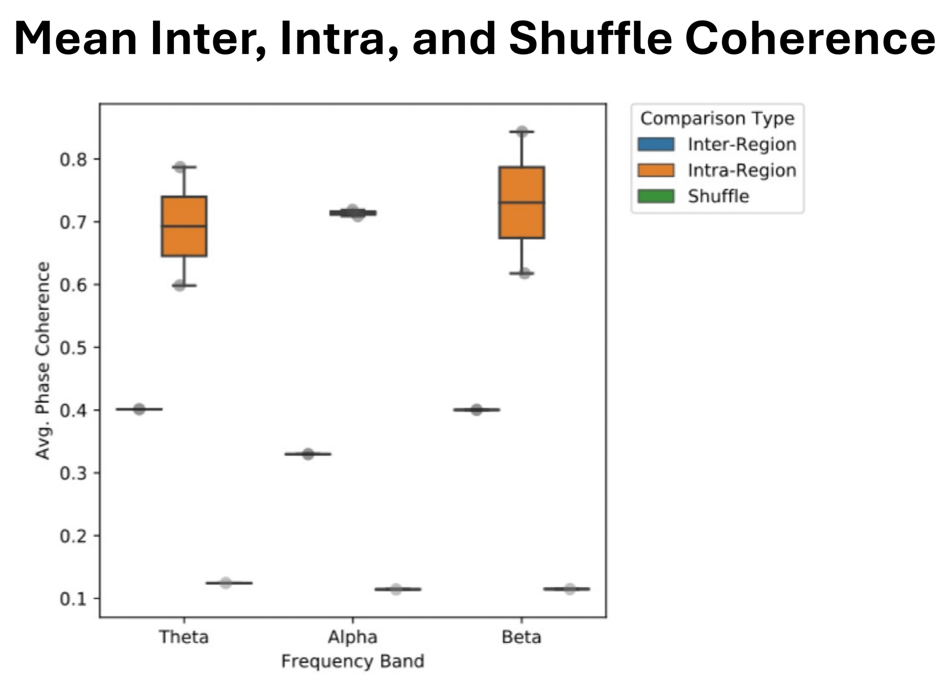


Figure 9. (Top Left) LFP Coherence before and after stimulus delivery at time 0 s for orthonasal olfaction. **(Top Right)** Similarly the retronasal coherence over time with the highlighted area in yellow being significantly increased levels in coherence post stimulus delivery. **(Bottom Left)** The fraction of significant correlations between GC and PC spikes during ortho and retronasal olfaction. The lines below indicate the 75% range of correlations you would get by shuffling the data. **(Bottom Right)** Comparison of different LFP coherence over different frequency ranges in GC and PC to Retronasally delivered odors; in comparison the second and third positions indicate intra-region coherence and coherence when the LFP signalsa are shuffled.

Changepoint Models for Orthonasal and Retronasal Olfaction

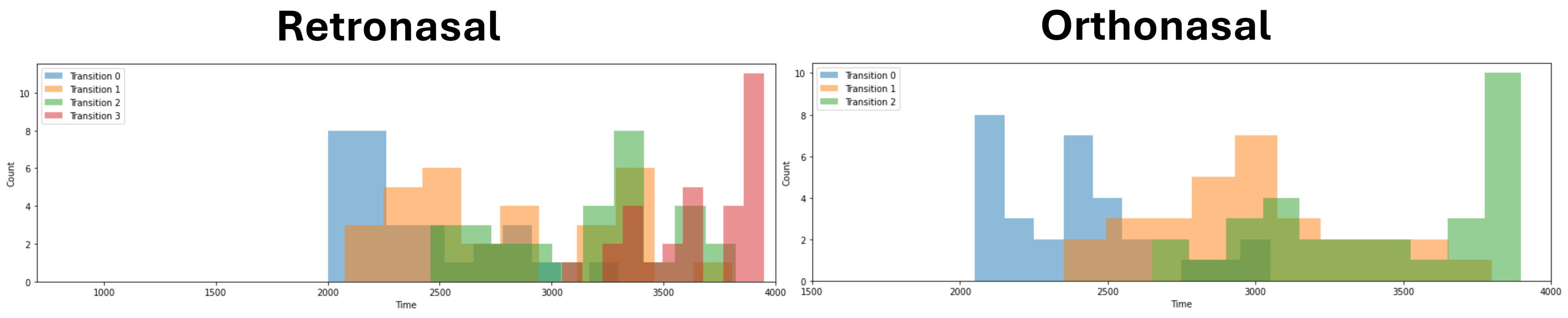


Figure 10. (Top Left) Solid lines demarcate a known connection and influence of one area on another. Dotted lines indicate an uncharacterized but predicted connection. **(Top Right)** Functional connectivity diagram of gustatory cortex (GC) and piriform cortex (PC) describing a previous experiment[1] where GC excitatory neurons were inhibited with optogenetics and PC neurons were recorded from with single channel electrodes. . **(Bottom Left)** . **(Bottom Right)**

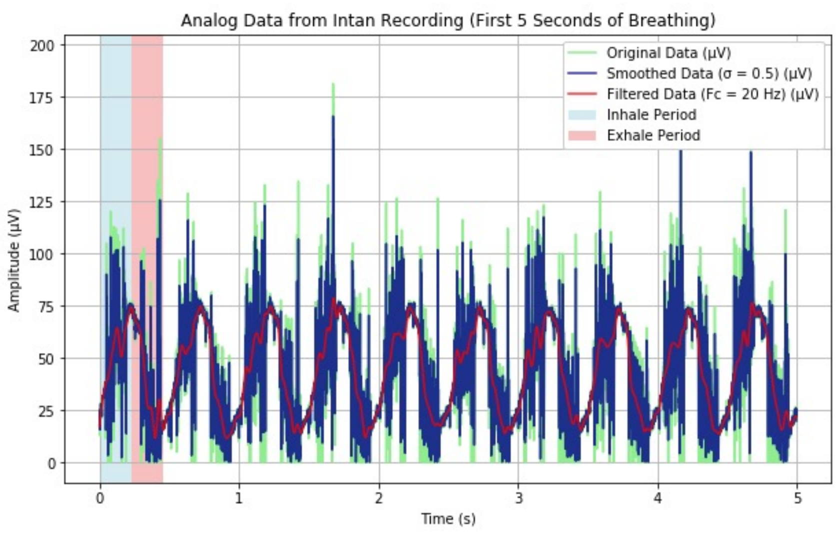
Conclusions

Specific Aim: Characterize the dynamic activity between Gustatory Cortex and Piriform Cortex in response to retronasal olfaction and orthonasal olfaction and identify whether there is phase coherence.

1. LFP from GC and PC become coherent over the alpha band during the odor response around the palatability epoch of GC specific to the retro mode.
2. The fraction GC-PC neuron pairs that significantly correlate in firing are higher than chance in retronasal olfaction.
3. Based on preliminary data, we suggest GC leads PC in responses to retro odors.

Future Directions

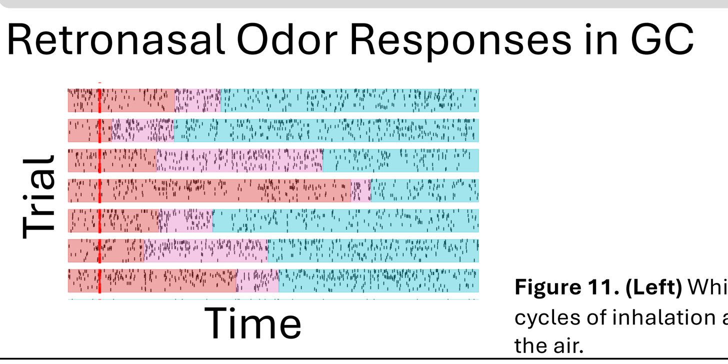
Alignment of Breathing to Electrophysiology



To correctly align the onset of stimulus we plan to reanalyze the data using inhalation as stimulus onset instead of the odor delivery to the animal.

Figure 11. (Left) While gathering data we simultaneously record the pressure changes via analog signal and transform it to cycles of inhalation and exhalation. We then use this to algin the odor delivery as when the animal inhales while the odor is in the air.

Hidden Markov Models for Ortho vs. Retro State Transitions



We plan to use Hidden Markov Models (HMMs) to find the state transitions along the odor response in GC and PC.

Figure 11. (Left) While gathering data we simultaneously record the pressure changes via analog signal and transform it to cycles of inhalation and exhalation. We then use this to algin the odor delivery as when the animal inhales while the odor is in the air.

Acknowledgements & Citations

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[1] Maier et al. 2015. doi: 10.1016/j.cub.2015.08.060.; [2] Blankenship et al., 2019. doi:10.1016/j.cub.2018.11.011; [3] Womelsdorf et al., 2007. DOI: 10.1126/science.1139597; [4] Mahmood et al. 2022 in Review